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TITLE: Psycho-Endocrine-Immune Profile: Implications for
Quality of Life in Breast Cancer Patients

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Table of Contents

Cover.....	1
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	12
Reportable Outcomes.....	12
Conclusions.....	13
References.....	13
Appendices.....	15

(1) INTRODUCTION

In the United States, approximately 1.5 million women undergo breast biopsy annually (1). Breast cancer diagnosis is a time of considerable uncertainty, anxiety, and emotional distress (2, 3). This emotional experience often begins with the discovery of clinical findings that indicate the need for biopsy of the breast. Women awaiting breast biopsy report higher levels of stress compared to patients awaiting general surgery (4). Reciprocal neuro-chemical pathways and shared receptor systems connect the nervous, endocrine, and immune systems (5, 6). This intricate communication network provides the link whereby perceived environmental stressors may effect the immune system and influence the course of disease (7). A large body of evidence has documented the impact of psychosocial stress on the human immune response (8). Several studies have examined the relationship between stress and natural killer cell activity (NKCA) and these studies have shown that stress can influence NKCA (9). Andersen *et al.* (10) studied the stress-immune response of women within four months of their breast cancer surgery but prior to adjuvant therapy initiation. The results of that study indicated that higher stress levels were predictive of lower NKCA, diminished natural killer (NK) cell response to interferon (IFN) gamma, and decreased lymphocyte proliferation (10). It is possible that stress may influence cancer control. Although a direct relationship between NKCA and cancer has not been equivocally established, patients with a variety of solid tumors (e.g., breast, cervix, endometrium, ovary, lung) do exhibit reduced NKCA (11). The effects of stress upon the immune system extend not just to NK cells but also to the production of cytokines. Heightened levels of stress are related to decreased synthesis of IFN γ (12) and a poorer NK response to IFN γ and interleukin-2 (IL-2) has been observed in stressed individuals compared to non-stressed individuals (13). Others have reported that posttraumatic stress disorder (14) and academic exam stress (15) also lead to cytokine dysregulation. Cytokine dysregulation in response to stress is believed to be triggered by an increase in adrenal cortisol secretion and a decrease in adrenal dehydroepiandrosterone sulfate (DHEAS) secretion (16). This shift in steroid hormone profile alters Th1/Th2 cytokine balance, leading to decreased production of IFN gamma (a Th1 cytokine) and enhanced production of IL-4 (a Th2 cytokine) (17). Such a change in cytokine balance can depress NK cell function (18). The purpose of this study is to investigate psychological stress and its' potential impact on the immune response (NKCA and cytokine balance) of women before and after breast biopsy. The design of this study allows the exploration of the biological links between stress and immune function at an early point in a woman's encounter with cancer.

(2) BODY

Methods - All women over 18 years of age, who seek consultation at the Breast Care Center of Loyola University's Cancer Center and who subsequently receive a breast biopsy (excluding fine needle aspirates) were eligible subjects. Exclusionary criteria include: pregnancy, prior (within 5 years) or current history of cancer, recent history of major psychiatric disorder or concurrent major immune-based disease, and active substance abuse. Women were studied at the following four time points:

- T₁ - Initial consultation at the Breast Care Center

- T₂ - Day of biopsy, prior to the actual biopsy
- T₃ - At return clinic visit, 10-14 days after notification of biopsy results
- T₄ - 1-2 months after T₃.

At each time period volunteers completed informed consent documents, had their blood drawn, and completed the psychological measures and a health history questionnaire.

Psychological Measures: Psychological stress was defined as appraisal and perception of an environmental event (i.e., breast biopsy and a possible cancer diagnosis) that is viewed as threatening an individual's sense of well-being. Stress perception leads to psycho-biological responses which are measurable.. The psychological measures included a visual analogue scale (VAS) for global stress and for biopsy-related stress, Cohen's Perceived Stressor Scale (PSS), Spielberger's State Trait Anxiety Inventory (STAI), and the Profile of Mood States (POMS).

Laboratory Measures: Serum was stored for the analysis of the stress hormones, cortisol and dehydroepiandrosterone/sulfate (DHEA/DHEAS), by radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA). Peripheral blood mononuclear cells (PBMC) were derived immediately by Ficoll/Hypaque separation. These isolated PBMC's were assessed for NKCA against [⁵¹Cr] labeled K562 tumor targets as described previously (20). Cytokine production was measured by stimulation of peripheral blood mononuclear cells in bulk culture. Culture supernatants were collected after 48 hrs and cytokines were measured using standard ELISA kits (R & D Systems, Minneapolis, MN).

Statement of Work To Date.

Task 1. Determine the psycho-endocrine and NK cell response of women to the experience of breast biopsy for cancer diagnosis (months 1-36). Twenty-two women have been enrolled in this study over the past year. The total number of women enrolled in this study to date is 130. Of the total women enrolled in this study, 16 women did not have a biopsy performed. This was often a clinical decision occurring on the day of biopsy prior to the actual procedure. Four women withdrew from the study. Of the 114 women undergoing breast biopsy, 84 had benign biopsy results (72.8%), while 30 women were diagnosed with malignancy (26.3%). The racial/ethnic distribution is as follow: White (N=107), African American (N=13), Hispanic (N=4), Asian (N=2), and Other/Unknown (N=4). The ages of subjects ranged from 18 to 85 years of age. The mean±SEM age of all subjects was 52.8±1.3 years (benign 50.4±1.4; malignant 60.3±2.3). Not all women were able to complete all four data collection periods; hence, the subject number varies per data collection time period. Also, some women chose not to complete some instruments. Forty six non-biopsied age-matched control women were assessed to establish psychological norms, but immune measures were done on only a subset of the control women. In order to provide the best indication of the results to date, the data that is reported herein is reported for the total number of women enrolled at this time. The psycho-endocrine stress profile and NK cell activity of each subject pre and post breast biopsy was measured. Each individual was administered psychological instruments, had blood drawn and peripheral blood mononuclear cells isolated for measure NK cell activity and plasma adrenocortical stress hormones (cortisol, DHEAS). Not all endocrine and cytokine assays have been completed on frozen serum samples. Relevant

demographic, medical history, and disease characteristics from medical records were abstracted and contact with subjects was maintained per telephone to ensure subject retention, and quality control of laboratory and psychological data collection has been accomplished. This study has been granted a no-cost extension to complete subject recruitment and final data analysis.

Task 2. Identify the importance of the Th1/Th2 cytokine profile to the psycho-endocrine-NK cell response of women undergoing breast biopsy for cancer diagnosis (month 1-36). For each of the blood samples from the above subject, cytokine production was measured pre and post biopsy.

Task 3. Understand differences in the psycho-endocrine-immune profile of women with benign versus malignant breast biopsy findings (months 24-36). Cytokine variables are presented for the all women and the stratification of this data into benign and malignant groups.

Task 4. Analysis and Manuscript Preparation is underway (months 24-36) one article has been published, one chapter has been published, and one article has been submitted.

Summary of Results

This study investigated psychological indices of stress and immunological parameters hypothesized to be affected by stress. Results for all figures are reported in the same general manner and all Figures are located in the **Appendix**. The mean psychological and immunological results are indicated for pre biopsy time points (T1 and T2) and post biopsy time points (T3 and T4). The time frame for these measures is defined above in Methods. At each time point, results are presented for all women who underwent biopsy as well as for subgroups of these same women who ultimately had either benign or malignant findings. The biopsy groups are the following: All: All women undergoing biopsy no matter what the biopsy findings; Benign: women with benign biopsy findings, and Malignant: women with malignant biopsy findings. For each time point (T1-T4) comparisons were made within the biopsy groups. In addition, between group comparisons were made to compare each biopsy group to a group of non-biopsied control women.

Psychological Assessments: Figure 1 illustrates results for perceived stress (PSS) for women at pre biopsy (T1 and T2) and at post biopsy (T3 and T4), as well as perceived stress for control (non-biopsied) women. The results indicate that the period of biopsy was a time of reported perceived stress. All three groups of women at T2 and T3 reported perceived stress that was significantly greater than that of the non-biopsied control women. In addition, the All and Benign groups at T1 and T4 reported significantly greater perceived stress than control women. For benign women at T1 a significant increase in perceived stress was observed when compared to the perceived stress reported by the same group of women at T2 and T4. Of note, the All and Benign groups showed a numerical reduction in perceived stress post biopsy (T3 and T4), but this reduction was not significant and the women reported levels of stress similar to pre biopsy. This was not the case for the malignant group of women.

In Figure 2, results are presented for the VAS (10-cm stress visual analogue scale), which indicates the current perception of overall stress in a woman's life. The results show that the two pre biopsy time points were times of psychological stress for all women. Global stress levels decreased at both

post biopsy time points in all three groups of women. The reductions in global stress were most marked for the benign women at T3; while stress in the malignant group was significantly greater than the benign group at this time. All three groups of women reported statistically significant elevations in global stress at T2 and for the All and Benign groups at T1, when compared to non-biopsied, control women. No differences were observed between reported stress by biopsied women and non-biopsied, control women at T3 or T4. At T1 and T2 the results for all biopsied women and the women with benign findings were statistically different from the reported results for T3 and T4. In contrast, T2 results for women who ultimately had malignant findings were only significantly different at T4.

Similar to the PSS, the POMS total mood disturbance score (POMS TMD) was significantly elevated for women pre biopsy compared to control, non-biopsied women, Figure 3. Self-reported total mood disturbance was significantly reduced pre to post biopsy, but the scores remained significantly elevated when compared to scores reported by control women. Exceptions were the scores reported at T4 by the women in the Malignant group, which did not differ from those of the control women. Similar to the PSS and the POMS-TMD were the scores reported for Anxiety. Anxiety was significantly elevated for women pre biopsy compared to control, non-stressed, women, Figure 4. Self-reported Anxiety was significantly reduced pre to post biopsy, but the scores remained significantly elevated when compared to scores reported by control women. Exceptions were the scores reported at T1 and T4 by the women in the malignant group, which did not differ from those of the control women. Of note, the scores for the malignant group of women were significantly higher at T3 than were the scores for women in the benign group.

The POMS is comprised of six subscales that measure Tension, Depression, Anger, Vigor, Fatigue, and Confusion. Figures 5-10 show changes over time for these mood subscales. In Figure 5 comparisons are presented pre biopsy to post biopsy and to control (non-biopsied) women for the Tensions subscale of the POMS. The results indicate that the period prior to biopsy was a time of reported tension. All three groups of women at T2 and the All and the Benign groups at T1 reported tension that was significantly greater than the control women did. This tension was significantly reduced after biopsy. However, for the All and the Malignant groups at T3 and the All and Benign groups at T4, the tension was still significantly elevated compared to control women.

In Figure 6 comparisons are presented pre biopsy to post biopsy and to control (non-stressed) women for the Depression subscale of the POMS. The results indicate that the period prior to biopsy was a time of reported depression. All three groups of women at T2 and the All and the Benign groups at T1 reported depression that was significantly greater than the non-stressed control women. This depression did not return to normal levels after biopsy, with the exception of the Malignant group at T4. The results of the POMS Anger subscale (POMS-A) indicate that the period prior to biopsy was a time of reported anger for some of the women (Figure 7). The All and Benign groups at T1 reported anger that was significantly greater than that of the control women. Anger returned to control levels after biopsy. Of note, however, the women in the Benign group showed significantly more anger than did the women in the Malignant group at T4.

As shown in Figure 8, the pre biopsy period was a time of reduced vigor (POMS-V). All three groups of women at T2 and T3 as well as the All and the Benign groups at T1 and T4 reported vigor

that was significantly less than the control women did. This decreased vigor did not return to normal levels after biopsy, with the exception of the Malignant group at T4. As for fatigue (POMS-F), the results are variable but indicate that for some women the period prior to biopsy was a time of increased fatigue (Figure 9). This was true for the All and Benign groups at T1 and for the All and Malignant groups at T2. After biopsy no differences in fatigue were noted compared to control women. Figure 10 illustrates that prior to biopsy women reported confusion (POMS-C). All three groups of women at T2 and the All and the Benign groups at T1 reported confusion that was significantly greater than the non-stressed, control women did. Confusion did not return to normal levels after biopsy for the All group at T3 or for the Malignant grouping at T3.

Immunological Assessments: The NKCA for PBMC (lytic units at 20%) of women pre and post breast biopsy is illustrated in Figure 11. In comparison to the control women, NKCA was significantly depressed for both the All and Benign groups for each time period, with the exception of the Benign grouping at T4. Significant depression in NKCA was only observed after biopsy (T3 and T4) for the Malignant group. Of note, the NKCA for both the All group and the Benign group were statistically greater than the NKCA for the Malignant group at T4. The NKCA of the Benign group at T4 was not statistically different from control women. Phenotypic analysis for circulating NK cells showed no differences between normal control subjects and biopsy patients at any time point (Figure 12).

Cytokine production by PBMC of women pre and post biopsy was evaluated as well. For IL-2 production, no significant changes were observed for any of the groups at any of the time periods. See Figure 13. Those results are quite dissimilar to those obtained for IL-6, shown in Figure 14. For all biopsied groups, at all time points, IL-6 production was significantly greater than the amount of IL-6 produced by control women. Only in one case, did a statistically significant reduction occur between time periods and that was for the Benign group of women at T2 in comparison to the same group at T4. In contrast to those results, the capacity to produce interferon (IFN) gamma was significantly reduced for the All groups at T2 and T3, and for the Benign grouping at T3, when the results were compared to control women (Figure 15). At all time points the quantity of produced IFN gamma was numerically less for the Malignant grouping than for any of the other two groups. Furthermore, the quantity of IFN gamma produced by the All and Benign groups were significantly less at T2 and at T3 when compared to the quantity produced at T4. Much like IL-6, increases IL-4 and IL-10 production were observed in the biopsied women compared to the control women. In comparison to control women, IL-4 production was significantly increased for all groups of biopsied women at T2 and for the Malignant grouping at T1 and T3 as well as for the All group at T3 (Figure 16). IL-4 production by all groups of women significantly decreased from T2 to T4, and by T4, IL-4 production was not different from control women. IL-10 production by the PBMC of biopsied women was elevated in comparison to control women for all three groups of biopsied women and for all time periods (Figure 17). No significant reductions in IL-10 were observed among time periods. No cytokines were detected in the serum of representative control or biopsy subjects. Data not shown.

Psychological Effects Upon Immune Function: Humans appraise and perceive events in an individualized manner and hence, the response to a potentially threatening event is also heterogeneous. In this sample of women, "response" to the experience of breast biopsy was

categorized as low, moderate, or high. For this analysis the psychological measures employed for this categorization were the PSS, Anxiety, and the POMS TMD. Categories of response were determined for each tool by placing the subject's responses at T1 into three categories as follows; a low response was defined as the bottom 25% of all responses, a moderate response was defined as the middle 50% of all responses, and a high response was defined as the top 25% of responses. The values used to define these categories of response were numerically as follows: PSS, low <13.0, moderate $\geq 13.0 - 22.5$, high > 22.5 ; Anxiety, low < 30.0, moderate $\geq 30.0 - 54.0$, high > 54.0 ; POMS TMD, low <8.0, moderate $\geq 8.0 - 52.0$, high > 52.0 . Subjects were grouped into low, moderate, and high categories for each of these psychological measures for each time period and means and SEM for each immunological measure were determined for each time period based upon the subject's report of low, moderate, or high responsiveness.

Women entered into the study were all aware that breast biopsy was impending and as described above, experienced perceived stress, anxiety, and mood disturbance at T1 in comparison to non-biopsied, control women. This psychological effect continued through T2 and in most cases through T3 and in some cases through T4. Reductions in NKCA were observed as early as T1 and continued through T3 for the three groups of women and for the All and Malignant groups of women through T4 (Figure 11), when compared to control women. At T2 a numerical increase in NKCA was observed in comparison to T1 and T3. This numerical increase in NKCA at T2 appears to be a consequence of an increase in NKCA for those individuals with the highest levels of perceived stress, anxiety, and total mood disturbance (Figure 18). In all cases, increases were numerical with the exception of the PSS. Statistically significant differences were observed between individuals with low perceived stress and the groups with moderate and the highest levels of perceived stress. Moreover, no difference in the percentage of circulating NK cells was observed in women who experienced low, moderate, or high perceived stress, anxiety, or mood disturbance (Figure 19). Hence, the observed effects at T2 were upon NKCA and not upon changes in numbers of circulating NK cells.

With regard to IL-2 the overall production of this cytokine was not different for any of the groups at any of the time periods examined in comparison to the control women (Figure 13). However, when women were placed into categories based on responses to the experience of biopsy, the women who experienced the most perceived stress, anxiety, and total mood disturbance, generally had the least numerical production of IL-2 (Figures 20 and 21). For T1 the reduction in IL-2 production was significantly different between the low and moderate mood disturbance categories and the high mood disturbance category. At T1, a significant difference was observed as well between the low anxiety category and the high anxiety category. Significant differences were observed between low and high anxiety at T4 and between low and moderate perceived stress. At T2 a similar pattern numerically was observed but did not reach statistical significance (Figure 22). At T3 no pattern was consistently recognizable. Data not shown. Of note, PSS and IL-2 production were negatively correlated ($r = -0.768$; $p \leq 0.05$) for women in the malignant group at T4.

In Figures 23 and 24, comparisons of these categories of subjects for IFN γ production are shown. At T2, significant differences were observed between the low and high categories of perceived stress and at T3 significant differences were observed between low and moderate categories of

perceived stress. Similar numerical differences were observed for the other psychological measures at T2 and T3 but these differences were not significant. No pattern was consistently observed at T1 or T4. Data not shown. Comparisons of this type for IL-4, IL-6, and IL-10 did not provide further insight, data not shown.

Impact of Malignancy, Its' Therapy, and Psychological Measures On Immune Function:

Malignancy and the associated treatment can impact immune function. In Figures 25-30 the effects of cancer treatment or no treatment upon immune function (respectively NKCA, production of IL-2, IL-6, IFN γ , IL-4, and IL-10) are compared for the group of malignant women. Cancer treatment was either radiotherapy and/or chemotherapy. It is clear from the figures that in this restricted set of women no reduction in immune function was observed for therapeutically treated women when compared to untreated women at T3 or T4. Furthermore, it is possible that malignancy in and of itself may increase perceived stress, total mood disturbance, and/or anxiety.

If true, it would be anticipated that a large percentage of the women with diagnosed malignancy would also report the highest levels of perceived stress, total mood disturbance, and/or anxiety. In Table 1 is presented the number of women with malignancy who reported low, moderate, and high levels for these psychological measures at T1, T2, T3, and T4. It is clear from this data that the number of women with malignancy was evenly distributed throughout the categories and did not cluster in the "high" categories at any of the time points.

Measurement of Hormonal Levels: Circulating levels of cortisol and DHEASO₄ were measured for biopsied women at T1, T2, T3, and T4. The data are presented in Figures 31 and 32. At this time, no significant differences are observed.

Discussion

In order to determine whether psychological stress accompanies breast biopsy, scores on various stress measures pre and post biopsy were compared to non-biopsied control women. The results clearly showed that women undergoing breast biopsy scored higher than did age-matched, non-biopsied, control women for all of the psychological measures evaluated. Moreover, pre biopsy subjects reported significantly more psychological stress, more perceived stress, experienced more anxiety, and more total mood disturbance, than did the subjects post biopsy. Collectively, these results demonstrate the experience of breast biopsy to be a psychologically stressful event. It is also noteworthy that, with the exception of the VAS, emotional distress and mood disturbance remained significantly elevated after biopsy. For the majority of women, the POMS-TMD and the Anxiety index (STAI) showed significant reduction pre biopsy to post biopsy, but this was not the case for the PSS, which remained statistically similar to pre biopsy scores. However, for most of the POMS subscales, values pre biopsy were still greater than those reported by control women. A significant reduction in scores was reported for tension, depression, and confusion pre to post biopsy. In the case of vigor, pre biopsy women reported less vigor than post biopsy women did and post biopsy women reported less vigor than non-biopsied, control women did. These data clearly show that the experience of breast biopsy produced psychological distress and mood disturbance in women that extended well beyond the day of biopsy. In particular, increased tension and depression and reduced vigor significantly contributed to the mood disturbance after biopsy with minor contribution by confusion.

Th1, Th2, and proinflammatory cytokine production fell into three distinct categories for the biopsy subjects. As a group, production of cytokines by the PBMCs of the biopsied women either remained unchanged (IL-2), showed an increase compared to control subjects (IL-4, IL-6, and IL-10), or alternatively showed a reduction when compared to control subjects (IFN gamma).

IL-2 has been characterized as a Th1 cytokine and falls into the first category. Its' production by the majority of biopsy patients (as a whole group) was unchanged from pre to post biopsy and was approximately the same as that of control subjects. IL-6 is a pro-inflammatory cytokine that falls into the second category. Its' production by all biopsy patients was greater than control subjects with increased levels of production, pre and post biopsy. In this category as well are IL-4 and IL-10, which are both Th2 cytokines that showed markedly increased production during the biopsy experience. IFN gamma, a Th1 cytokine, falls into the third category and showed decreased production by the majority of biopsy patients in comparison to control subjects. In addition to these measures, a marked reduction was observed in NKCA between control, non-biopsied, women and women who experienced biopsy. Women entered into the study were aware that breast biopsy was impending and as described above, experienced psychological stress pre biopsy. The impact upon NKCA appeared early (T1) and continued throughout the post biopsy period. The pre biopsy time points showed a significant diminution in mean NKCA for the majority of women. Because the reduction in NKCA was not accompanied by a change in the number of circulating NK cells these data clearly suggest that the observed reduction in NKCA is a consequence of reduced NKCA and not a reduction in NK cell number. As noted above significant emotional distress and mood disturbance was observed in women post biopsy when compared to that of control women. In sum, these results suggest that this distress appears to have, in turn, produced long-term effects on immune function; given that reduced NKCA and dysregulation in cytokine production continued beyond the day of biopsy.

Intriguing immunological differences were observed when the psychological response to breast biopsy was stratified as low, moderate, and high. With regard to NKCA, a numerical increase in NKCA was observed at T2 relative to T1 and to T3. This numerical increase appears to be a consequence of enhanced NKCA at T2 by the women who experienced the greatest amount of perceived stress, anxiety, and total mood disturbance. Moreover, it seems likely that the actual day of biopsy is a period of heightened psychological distress in that the highest scores for the stress (VAS), anxiety (STAI), and the POMS tension and confusion subscales were reported at T2. T2 was also the only time period when all reported psychological measures were greater for all groups of women compared to controls (with the exception of anger). At T2, NKCA was significantly higher in individuals with moderate and the highest levels of perceived stress in comparison to those with the lowest level of perceived stress. Likewise, production of IL-6 and IL-10 were greatest at T2. For IL-2 production, when analyzed as a group, no significant differences were observed between any of the time points for biopsied women compared to control women. However, at T2 (and also at T1 and T4) the women with the greatest psychological effect had the least production of IL-2.

It is important to note that the "biopsy experience" involves a relatively longer period of potential stress and uncertainty from discovery of a abnormal breast findings to learning the results of a biopsy. This may extend for several weeks in some cases. Layered upon this "chronic" stress is the actual day of biopsy, which can be viewed as an acute episodic stressor. With this in mind,

the acute effects of impending breast biopsy (at T2) enhanced NKCA and affected cytokine production in a directional manner. The acute experience of biopsy augmented NKCA for the most psychologically affected individuals. Blood was drawn and NKCA measured immediately prior to breast biopsy. Under these circumstances, the acute effect was observed as an enhancement in NKCA. In contrast, the acute effect of breast biopsy augmented the overall directional effects of the chronic experience of breast biopsy. In that, the overall effect on cytokine production at T1 and T3 was magnified at T2. Cytokine production was measured after 48 hours of culture of the acutely affected PBMC, which is significantly different from the immediate assessment of NKCA. Such a difference in assessment of immune function may explain the enhanced, directional cytokine responsiveness (IFN gamma production was further reduced and IL-4 and IL-10 production were further increased) to the acute experience of breast biopsy at T2. These results suggest that the day of biopsy is in fact a period of acute perceived stress, anxiety, and mood disturbance, and that this acute response may overlay the "chronic" effects of the experience of breast biopsy. Others have also described unique differences in immune response to chronic versus acute stressors (19).

It is worth noting that at T4 NKCA remained decreased for the women with malignant breast findings; whereas, in women with benign breast findings, NKCA increased to levels similar to control women. Our preliminary results suggest that the decrease in NKCA is not due to the cancer treatment therapy that the malignant women received. In addition, the decrease in IFN gamma observed at T2 and T3 may be a conservative estimate of the overall effect of distress upon this component of the immune system. The observed decrease may be limited by an overall maximal basal reduction, which is sometimes described as the "basement effect", in which no further reduction is observable due to the inhibitory nature of the biological effect. Moreover, IFN gamma production was most markedly reduced for the women in the malignant group and least affected for the women in the benign group. This difference could not be ascribed to cancer treatment therapy and for both groups of women, production of IFN gamma returned to normal levels by T4.

(3) KEY RESEARCH ACCOMPLISHMENTS

- A psycho-immune assessment of women at two time periods pre and two time periods post breast biopsy has been accomplished.
- Stress, perceived stress, anxiety, and mood disturbance are heightened in women pre biopsy and in some cases begin to "normalize" post biopsy.
- Perceived stress, anxiety, and mood disturbance diminish post biopsy but remain elevated in comparison to non-biopsied, control women.
- NK cell activity is depressed in women both pre and post breast biopsy compared to non-biopsied control women.
- Cytokine dysregulation accompanies the depression in NK cell activity.
- No changes in plasma cortisol or DHEAS were observed.

(4) REPORTABLE OUTCOMES (copies are in the Appendix)

Witek-Janusek, L., and H.L. Mathews. Stress, Immunity, and Health. 2000. In: *Handbook of*

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(5) CONCLUSIONS

These results provide evidence that the stress of breast cancer diagnosis leads to prolonged periods of perceived stress, anxiety, and mood disturbance, which appear to be associated with depressed NK cell activity and an altered pattern of cytokine production. Stress-induced alterations in immunity are not transient but persist beyond the acute experience of the biopsy. This may be of particular relevance to women diagnosed with malignancy since they will be facing additional stressors related to cancer treatment and adaptation to illness. This data set supports the need to adequately assess women to determine their risk of stress-related immune dysfunction and to incorporate stress-reduction strategies and to provide emotional support to women during the earliest stages of the cancer trajectory. Innovative approaches to buffer the effects of stress during cancer diagnosis would provide a valuable addition to comprehensive cancer care. Multi-disciplinary approaches to cancer diagnosis and treatment should seriously consider the incorporation of such approaches in the holistic care of women facing a cancer diagnosis. As these data support, such approaches might prove to be beneficial to both the psychological and immunological status of women with cancer. The treatment of women with breast cancer would benefit by the conduct of future studies that develop and evaluate biobehavioral approaches that promote optimal psychological and immunological well-being of these women.

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(7) APPENDICES

Figures and Legends to Figures

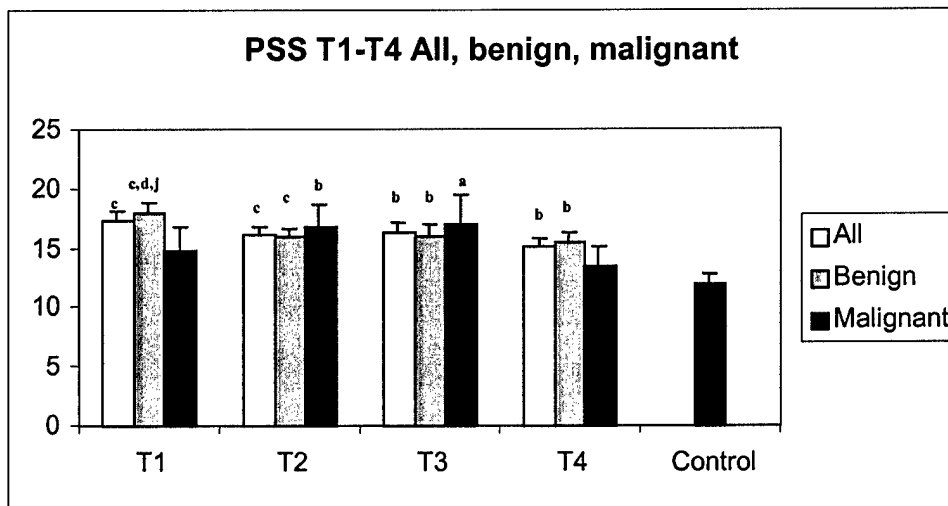


Figure 1. Psychological measure of perceived stress is depicted for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. Perceived stress was measured using Cohen's Perceived Stressor Scale (PSS). N for; All at T1= 82, All at T2= 94, All at T3= 77, All at T4= 81, Control=45. Benign at T1= 64, Benign at T2= 74, Benign at T3= 57, Benign at T4= 64. Malignant at T1= 18. Malignant at T2= 20, Malignant at T3= 20, Malignant at T4= 17. Bars represent the mean values \pm S.E. Statistical comparisons are as follows. a= $p \leq 0.05$, b= $p \leq .01$, c= $p \leq 0.001$ in comparing the respective experimental mean to the control group; d= $p \leq 0.05$ comparing benign biopsy group T1 to T2; j= $p \leq 0.05$ comparing benign biopsy group T1 to T4.

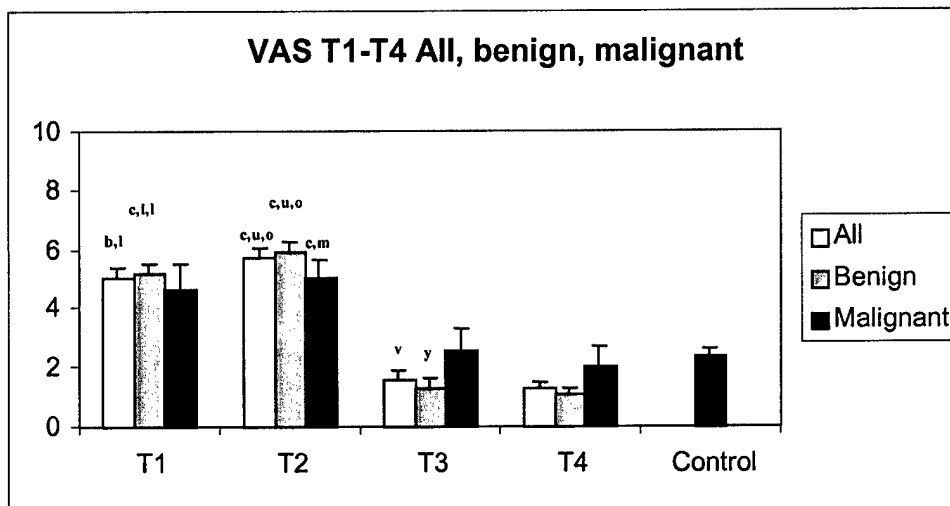


Figure 2. Psychological measure of stress is depicted for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. Stress was measured by use of 10 cm visual analogue scales that determined global stress (VAS). N for; All at T1= 82, All at T2= 94, All at T3= 77, All at T4= 81, Control=45. Benign at T1= 65, Benign at T2= 75, Benign at T3= 58, Benign at T4= 64. Malignant at T1= 17, Malignant at T2= 19, Malignant at T3= 19, Malignant at T4= 17. Bars represent the mean values \pm S.E. Statistical comparisons are as follows: a= $p \leq 0.05$, b= $p \leq .01$, c= $p \leq 0.001$ comparing the respective experimental mean to control group; i= $p \leq 0.001$ comparing T1 to T3 for biopsied women; l= $p \leq 0.001$ comparing T1 to T4 for biopsied women; m= $p \leq <0.0$ comparing T2 to T4 for biopsied women; o= $p \leq 0.001$ comparing T2 to T4 for biopsied women; u= $p \leq 0.001$ comparing T2 to T3 for biopsied women; v= $p \leq <0.05$ comparing All biopsied women to the malignant group at T3; and y= $p \leq .01$ comparing women with benign findings to women with malignant findings at T3.

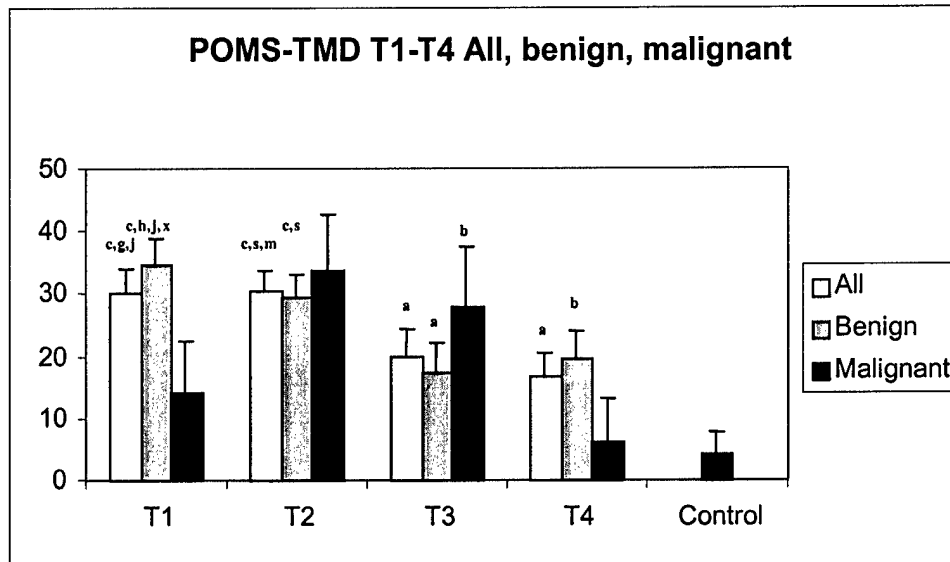


Figure 3. Psychological measure of mood state is depicted for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. Mood state was measured using the Profile of Mood States (POMS) and the total mood disturbance (TMD) is depicted. N for; All at T1= 79, All at T2= 92, All at T3= 79, All at T4= 85, Control=46. Benign at T1= 62, Benign at T2= 73, Benign at T3= 59, Benign at T4= 67. Malignant at T1= 17, Malignant at T2= 19, Malignant at T3= 20, Malignant at T4= 18. Bars represent the mean values \pm S.E. Statistical comparisons are as follows: a= $p \leq 0.05$, b= $p \leq .01$, c= $p \leq 0.001$ comparing the respective experimental mean to the control group; g= $p \leq 0.05$ comparing T1 to T3 for biopsied women; h= $p \leq .01$ comparing T1 to T3 for biopsied women; j= $p \leq 0.05$ comparing T1 to T4 for biopsied women; m= $p \leq 0.05$ comparing T2 to T4 for biopsied women; s= $p \leq 0.05$ comparing T2 to T3 for biopsied women; and x= $p \leq 0.05$ comparing women with benign findings to women with malignant findings at the respective time point(s).

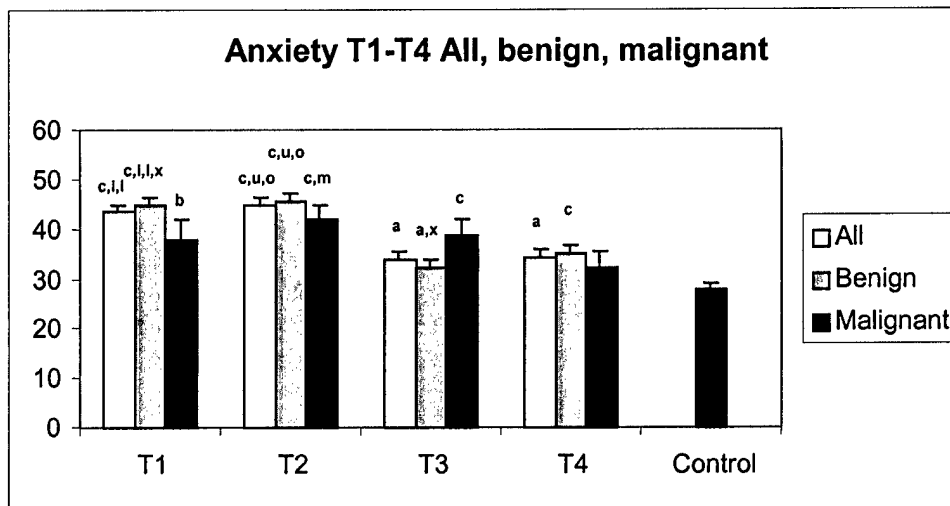


Figure 4. Psychological measure of anxiety is depicted for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. Anxiety was measured using Spielberger's State Anxiety Inventory (STAI). N for; All at T1= 83, All at T2= 96, All at T3= 78, All at T4= 83, Control=25. Benign at T1= 66, Benign at T2= 72, Benign at T3= 58, Benign at T4= 65. Malignant at T1= 17, Malignant at T2= 24, Malignant at T3= 20, Malignant at T4= 18. Bars represent the mean values \pm S.E. Statistical comparisons are as follows. a= $p \leq 0.05$, b= $p \leq 0.01$, c= $p \leq 0.001$ comparing the respective experimental mean to the control group; i= $p \leq 0.001$ comparing T1 to T3 for biopsied women; l= $p \leq 0.001$ comparing T1 to T4 for biopsied women; o= $p \leq 0.001$ comparing T2 to T4 for biopsied women; u= $p \leq 0.0$ comparing T2 to T3 for biopsied women; and x= $p \leq 0.05$ comparing women with benign findings to women with malignant findings at the respective time points.

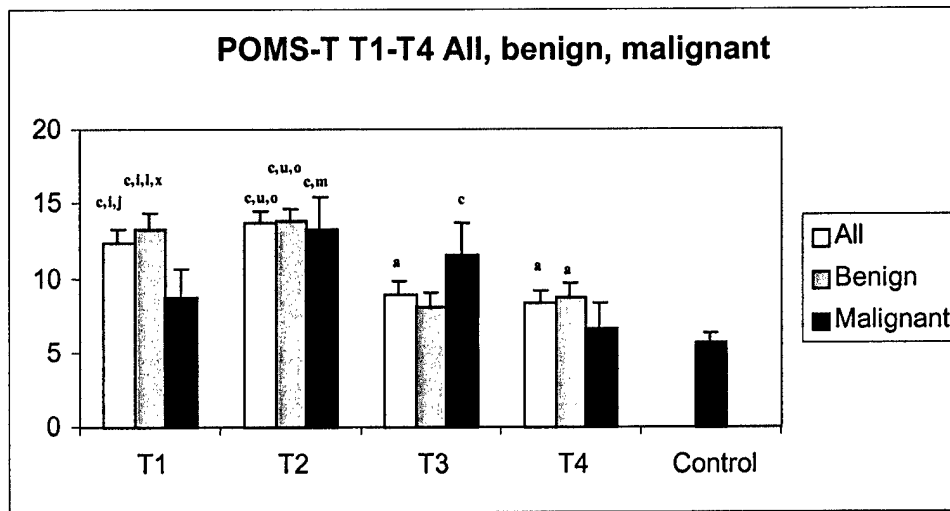


Figure 5. The tension subscale of the POMS is depicted for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. N for; All at T1= 79, All at T2= 94, All at T3= 79, All at T4= 85, Control=46. Benign at T1= 63, Benign at T2= 75, Benign at T3= 59, Benign at T4= 67. Malignant at T1= 16, Malignant at T2= 19, Malignant at T3= 20, Malignant at T4= 18. Bars represent the mean values \pm S.E. Statistical comparisons are as follows: a= $p \leq 0.05$, b= $p \leq .01$, c= $p \leq 0.001$ comparing the respective experimental mean to the control group; i= $p \leq 0.001$ comparing T1 to T3 for biopsied women; j= $p \leq 0.05$ comparing T1 to T4 for biopsied women; l= $p \leq 0.001$ comparing T1 to T4 for biopsied women; m= $p \leq 0.05$ comparing T2 to T4 for biopsied women; o= $p \leq 0.001$ comparing T2 to T4 for biopsied women; u= $p \leq 0.001$ comparing T2 to T3 for biopsied women; and x= $p \leq 0.05$ comparing women with benign findings to women with malignant findings at the respective time point(s).

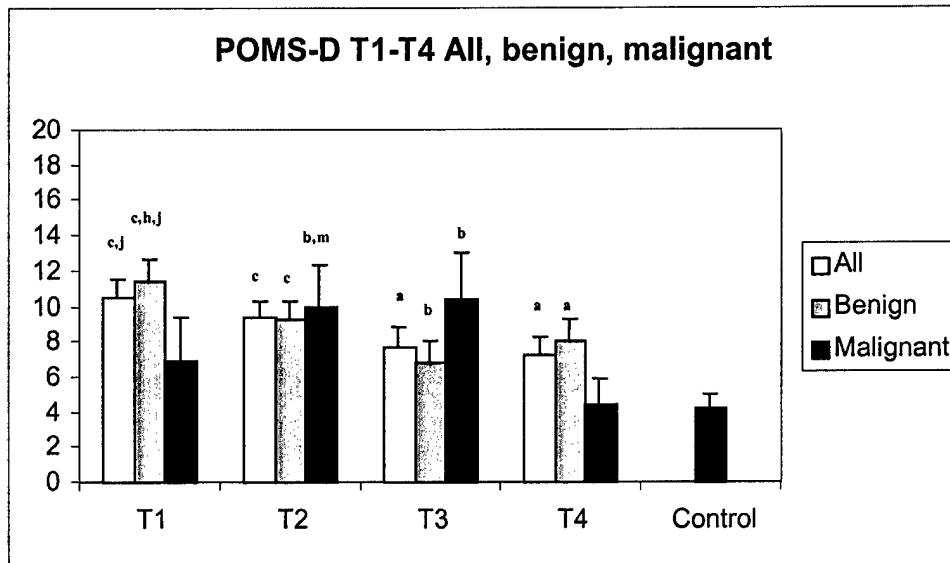


Figure 6. The depression subscale of the POMS is depicted for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. N for; All at T1= 78, All at T2= 92, All at T3= 79, All at T4= 84, Control=46. Benign at T1= 62, Benign at T2= 73, Benign at T3= 59, Benign at T4= 66. Malignant at T1= 16, Malignant at T2= 19, Malignant at T3= 20, Malignant at T4= 18. Bars represent the mean values \pm S.E. Statistical comparisons are as follows: a= $p \leq 0.05$, b= $p \leq .01$, c= $p \leq 0.001$ comparing the respective experimental mean to the control group; h= $p \leq .01$ comparing T1 to T3 for biopsied women; j= $p \leq 0.05$ comparing T1 to T4 for biopsied women; and m= $p \leq 0.05$ comparing T2 to T4 for biopsied women

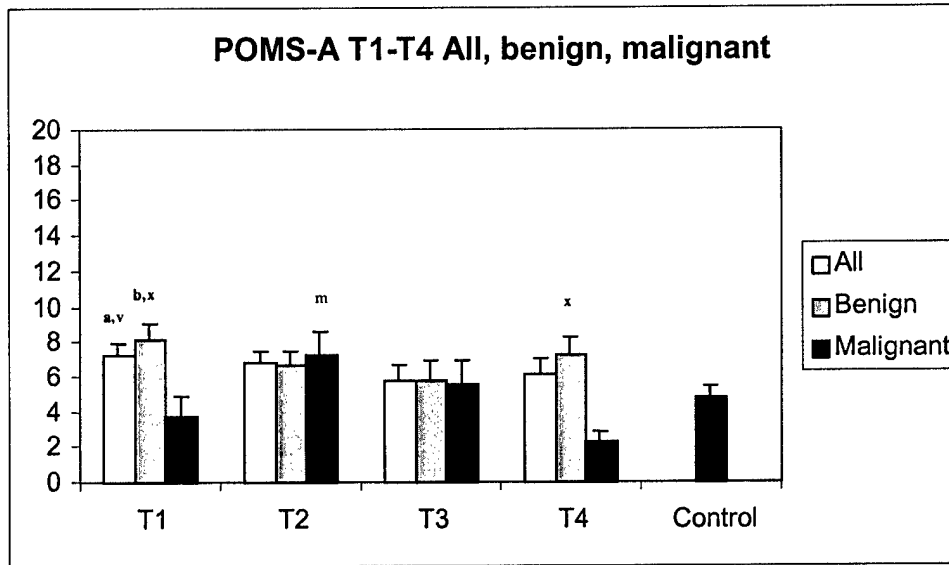


Figure 7. The anger subscale of the POMS is depicted for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. N for; All at T1= 79, All at T2= 92, All at T3= 79, All at T4= 85, Control=46. Benign at T1= 62, Benign at T2= 73, Benign at T3= 59, Benign at T4= 67. Malignant at T1= 17, Malignant at T2= 19, Malignant at T3= 20, Malignant at T4= 18. Bars represent the mean values \pm S.E. Statistical comparisons are as follows: a= $p \leq 0.05$, b= $p \leq 0.01$, c= $p \leq 0.001$ comparing the respective experimental mean to the control group; m= $p \leq 0.05$ comparing T2 to T4 for the biopsied women; v= $p \leq 0.05$ comparing All biopsied women to women with malignant findings at T1; and x= $p \leq 0.05$ comparing All biopsied women to women with malignant findings at the respective time point.

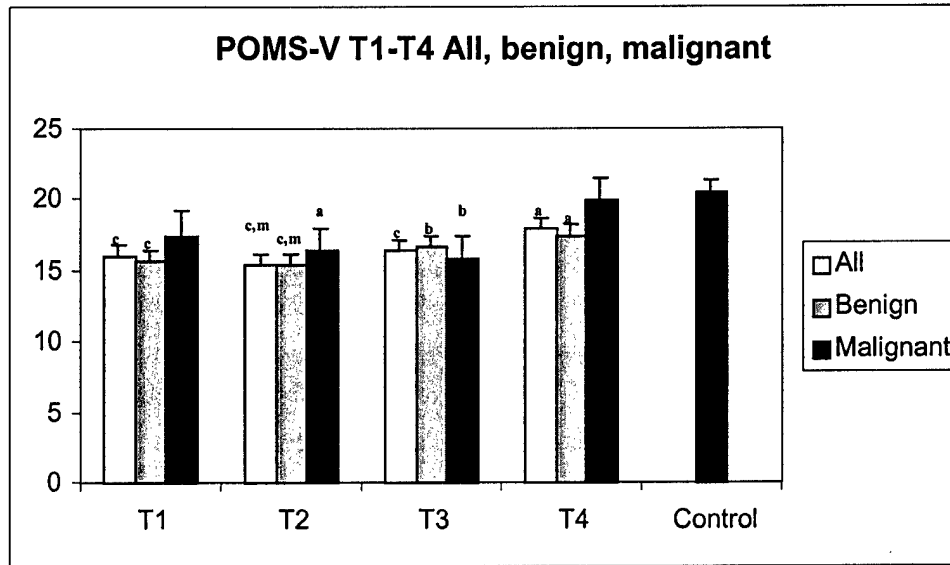


Figure 8. The vigor subscale of the POMS is depicted for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. N for; All at T1= 78, All at T2= 92, All at T3= 79, All at T4= 84, Control=46. Benign at T1= 61, Benign at T2= 72, Benign at T3= 58, Benign at T4= 66. Malignant at T1= 17, Malignant at T2= 20, Malignant at T3= 21, Malignant at T4= 18. Bars represent the mean values \pm S.E. Statistical comparisons are as follows: a= $p \leq 0.05$, b= $p \leq .01$, c= $p \leq 0.001$ comparing the respective experimental mean to the control group; and m= $p \leq 0.05$ comparing T2 to T4 for the biopsied women.

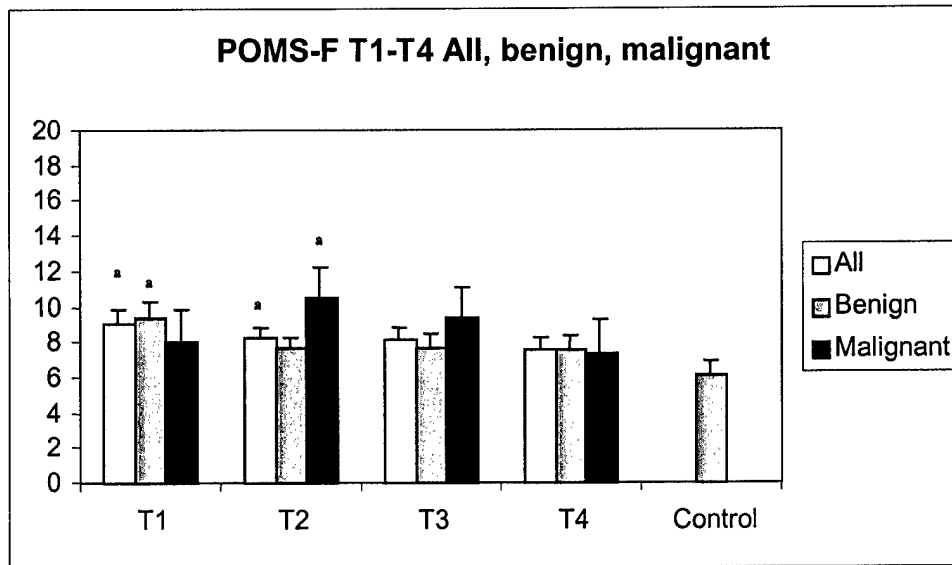


Figure 9. The fatigue subscale of the POMS is depicted for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. N for; All at T1= 78, All at T2= 92, All at T3= 79, All at T4= 85, Control=46. Benign at T1= 62, Benign at T2= 73, Benign at T3= 59, Benign at T4= 64. Malignant at T1= 16, Malignant at T2= 19, Malignant at T3= 20, Malignant at T4= 18. Bars represent the mean values \pm S.E. Statistical comparisons are as follows: a= $p \leq 0.05$, b= $p \leq .01$, and c= $p \leq 0.001$ comparing the respective experimental mean to the control group.

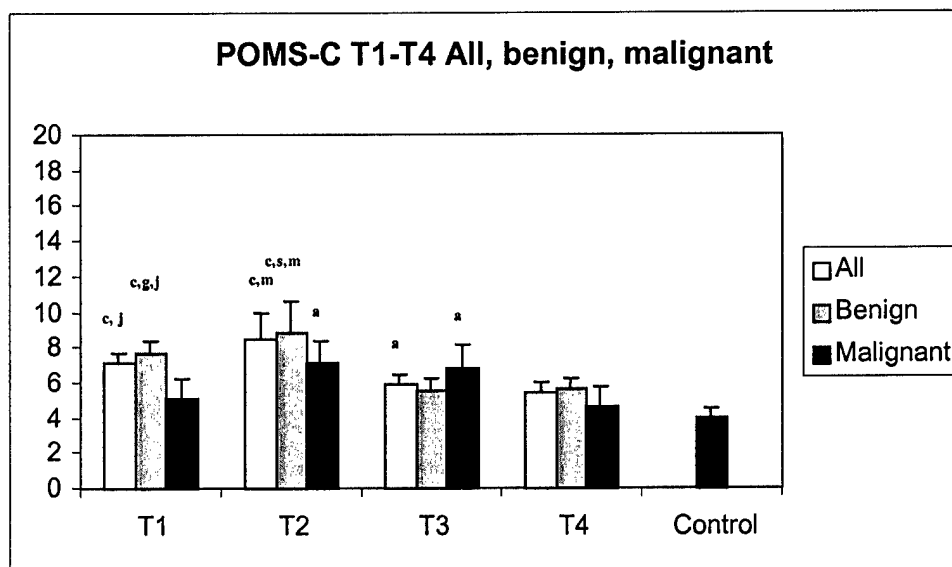


Figure 10. The confusion subscale of the POMS is depicted for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. N for; All at T1= 79, All at T2= 92, All at T3= 79, All at T4= 84, Control=46. Benign at T1= 62, Benign at T2= 73, Benign at T3= 59, Benign at T4= 66. Malignant at T1= 17, Malignant at T2= 19, Malignant at T3= 20, Malignant at T4= 18. Bars represent the mean values \pm S.E. Statistical comparisons are as follows: a= $p \leq 0.05$, b= $p \leq .01$, c= $p \leq 0.001$ comparing the respective experimental mean to the control group; g= $p \leq 0.05$ comparing T1 to T3 for biopsied women; j= $p \leq 0.05$ comparing T1 to T4 for biopsied women; m= $p \leq 0.05$ comparing T2 to T4 for biopsied women; and s= $p \leq 0.05$ comparing T2 to T3 for biopsied women.

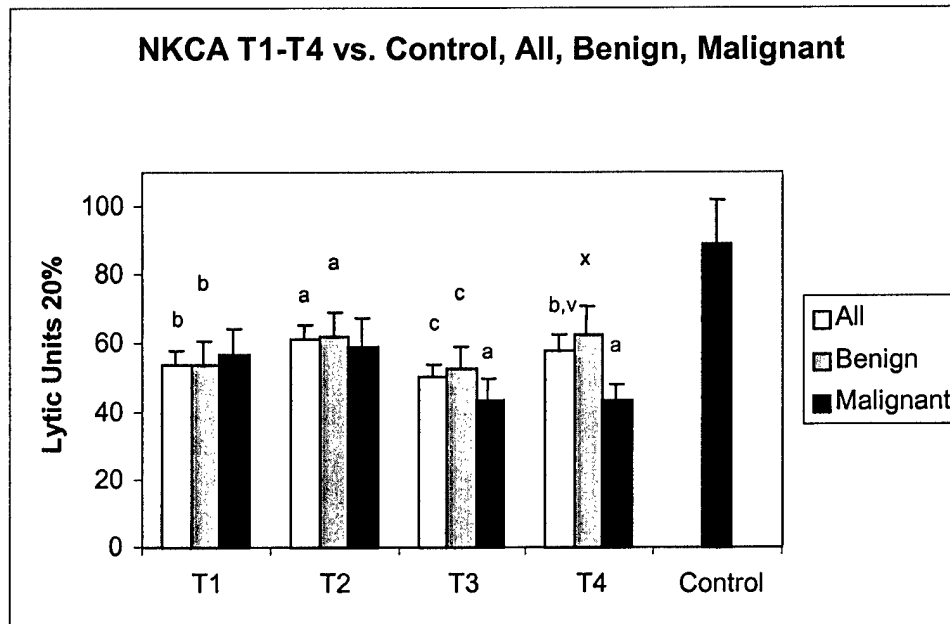


Figure 11. NKCA, expressed as lytic units at 20%, is illustrated for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. Peripheral blood was collected pre and post breast biopsy and from control women. NKCA was measured using K562 tumor cells as the target. N for; All at T1= 79, All at T2= 102, All at T3= 83, All at T4= 80, Control=14. Benign at T1= 64, Benign at T2= 76, Benign at T3= 63, Benign at T4= 62. Malignant at T1= 16, Malignant at T2= 26, Malignant at T3= 21, Malignant at T4= 19. Bars represent the mean values \pm S.E. Statistical comparisons are as follows: a= $p \leq 0.05$, b= $p \leq .01$, c= $p \leq 0.001$ comparing the respective experimental mean to the control group; v= $p \leq 0.05$ comparing All biopsied women (T4) to women with malignant findings at T4; and x= $p \leq 0.05$ comparing All biopsied women (T4) to women with malignant findings at T4.

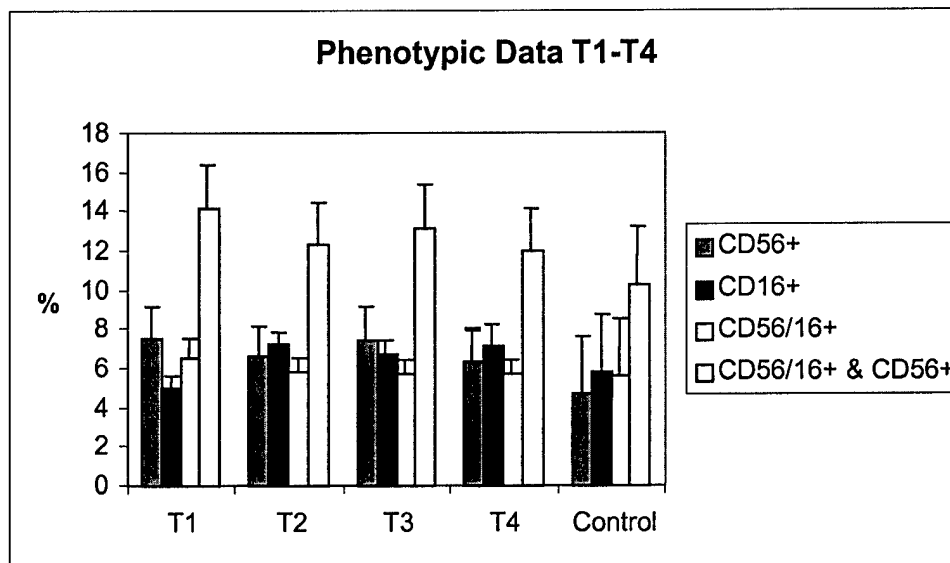


Figure 12. Phenotypic analysis of PBMC for all biopsied women. N for; T1 CD56+ = 26, CD16+ = 26, CD56/16+ = 26, CD56/16 & CD56+ = 26, T2 CD56+ = 31, CD16+ = 31, CD56/16+ = 31, CD56/16 & CD56+ = 31, T3 CD56+ = 29, CD16+ = 29, CD56/16+ = 29, CD56/16 & CD56+ = 29, T4 CD56+ = 23, CD16+ = 23, CD56/16+ = 23, CD56/16 & CD56+ = 23, T1 Control CD56+ = 8, T2 Control CD16+ = 8, T3 Control CD56/16+ = 8, T4 Control CD56/16+ & CD56+ = 8.

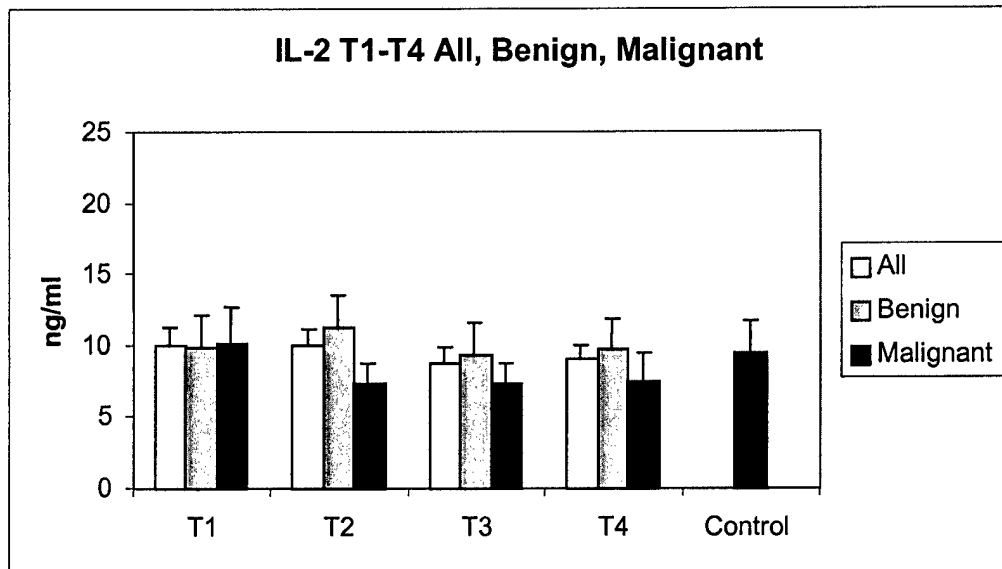


Figure 13. PBMC production of IL-2 is depicted for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. Peripheral blood was collected pre and post breast biopsy and from control women. PBMC were activated with PMA/PHA and culture supernatants were collected at 48 hr. Cytokine concentration was determined by ELISA. N for; All at T1= 52, All at T2= 63, All at T3= 51, All at T4= 53, Control=19. Benign at T1= 40, Benign at T2= 44, Benign at T3= 37, Benign at T4= 37. Malignant at T1= 12, Malignant at T2= 19, Malignant at T3= 14, Malignant at T4= 17. Bars represent the mean values \pm S.E. No statistical differences were found either within or between groups.

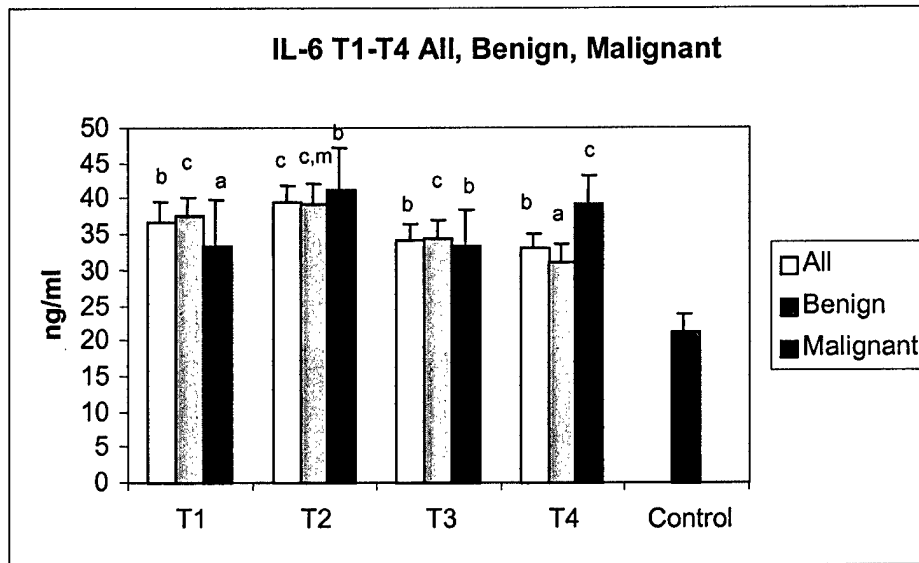


Figure 14. PBMC production of IL-6 is depicted for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. Peripheral blood was collected pre and post breast biopsy and from control women. PBMC were activated with PMA/PHA and culture supernatants were collected at 48 hr. Cytokine concentration was determined by ELISA. N for; All at T1= 59, All at T2= 78, All at T3= 69, All at T4= 60, Control=26. Benign at T1= 48, Benign at T2= 58, Benign at T3= 53, Benign at T4= 46. Malignant at T1= 10, Malignant at T2= 19, Malignant at T3= 16, Malignant at T4= 14. Bars represent the mean values \pm S.E. Statistical comparisons are as follows: a= $p \leq 0.05$, b= $p \leq .01$, c= $p \leq 0.001$ comparing the respective experimental mean to the control group; and m= $p \leq 0.05$ comparing T2 to T4 for biopsied women.

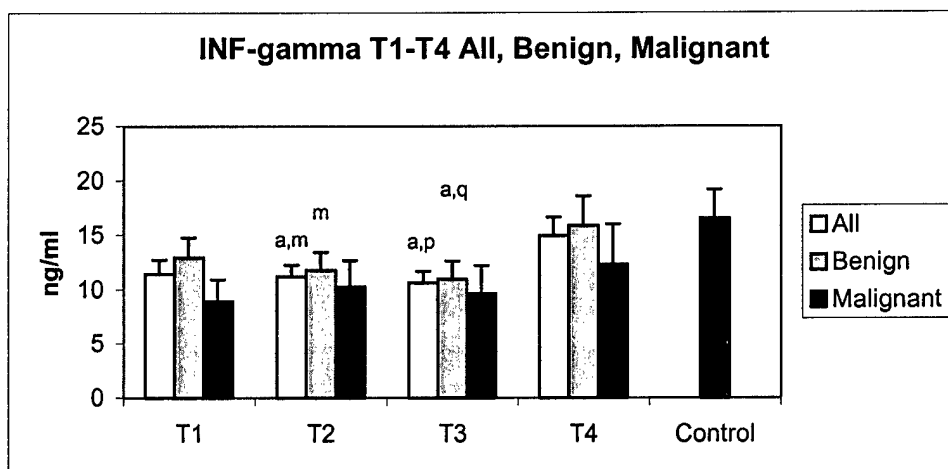


Figure 15. PBMC production of IFN γ is depicted for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. Peripheral blood was collected pre and post breast biopsy and from control women. PBMC were activated with PMA/PHA and culture supernatants were collected at 48 hr. Cytokine concentration was determined by ELISA. N for; All at T1= 64, All at T2= 82, All at T3= 64, All at T4= 49, Control=21. Benign at T1= 51, Benign at T2= 55, Benign at T3= 46, Benign at T4= 36. Malignant at T1= 14, Malignant at T2= 22, Malignant at T3= 18, Malignant at T4= 13. Bars represent the mean values \pm S.E. Statistical comparisons are as follows: a=p \leq 0.05, b=p \leq .01, and c=p \leq 0.001 comparing the respective experimental mean to the control group; m= p \leq 0.05 comparing T2 to T4 for the biopsied women; p=p \leq 0.05 and q= p \leq .01 comparing T3 to T4 for the biopsied women.

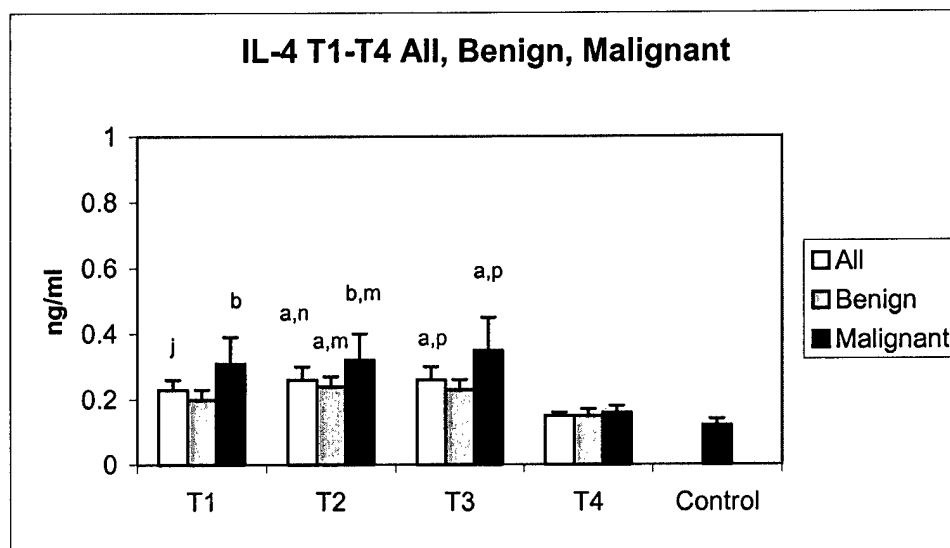


Figure 16. PBMC production of IL-4 is depicted for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. Peripheral blood was collected pre and post breast biopsy and from control women. PBMC were activated with PMA/PHA and culture supernatants were collected at 48 hr. Cytokine concentration was determined by ELISA. N for; All at T1= 64, All at T2= 80, All at T3= 73, All at T4= 77, Control=18. Benign at T1= 49, Benign at T2= 61, Benign at T3= 56, Benign at T4= 56. Malignant at T1= 15, Malignant at T2= 17, Malignant at T3= 17, Malignant at T4= 21. Bars represent the mean values \pm S.E. Statistical comparisons are as follows: $a=p \leq 0.05$, $b=p \leq .01$, and $c=p \leq 0.001$ comparing the respective experimental mean to the control group; $m= p \leq 0.05$ and $n=p \leq .01$ comparing T2 to T4 for the biopsied women; and $p= p \leq 0.05$ comparing T2 to T4 for biopsied women.

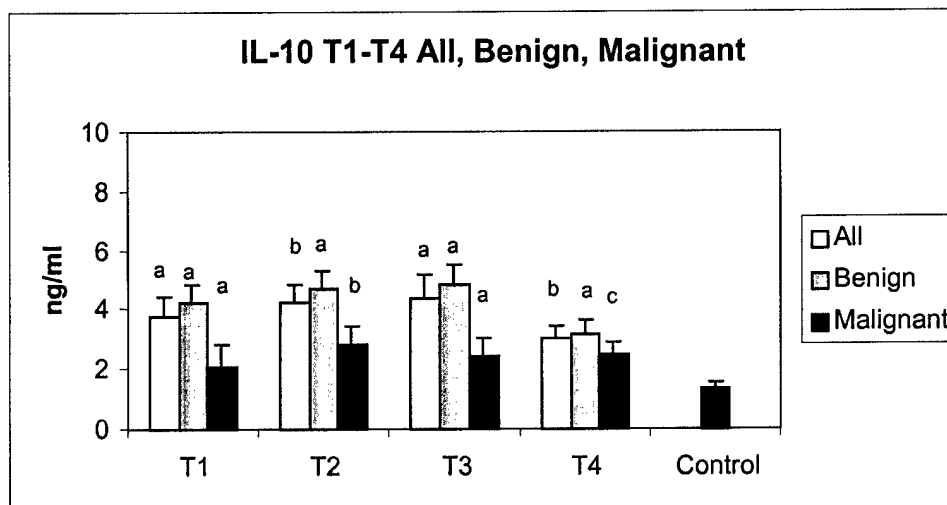


Figure 17. PBMC production of IL-10 is depicted for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. Peripheral blood was collected pre and post breast biopsy and from control women. PBMC were activated with PMA/PHA and culture supernatants were collected at 48 hr. Cytokine concentration was determined by ELISA. N for; All at T1= 62, All at T2= 81, All at T3= 68, All at T4= 65, Control=28. Benign at T1= 49, Benign at T2= 61, Benign at T3= 54, Benign at T4= 49. Malignant at T1= 13, Malignant at T2= 20, Malignant at T3= 14, Malignant at T4= 16. Bars represent the mean values \pm S.E. Statistical comparisons are as follows: a= $p \leq 0.05$, b= $p \leq .01$, and c= $p \leq 0.001$ comparing the respective experimental mean to the control group.

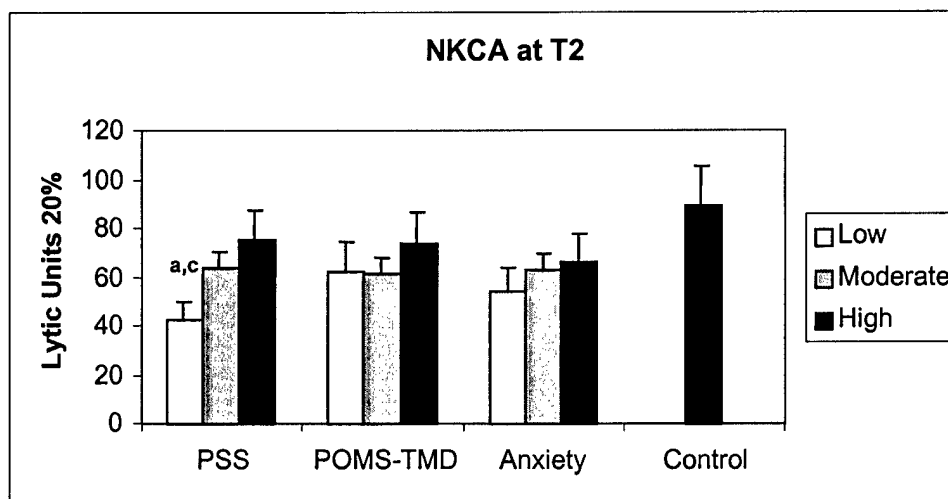


Figure 18. NKCA, expressed as lytic units at 20%, is illustrated for benign biopsied women at T2 who had low, moderate, or high levels of either perceived stress, total mood disturbance, or anxiety, and in comparison to non-biopsied control women. Peripheral blood was collected pre and post breast biopsy and from control women. NKCA was measured using K562 tumor cells as the target. N for; PSS for low= 19, moderate= 38, high= 17. Control=14. POMS-TMD for low= 18 , moderate= 39 , high= 15 . Anxiety for low= 17 , moderate= 41 , high = 16. Bars represent the mean values \pm S.E. Statistical comparisons are as follows: a= $p \leq 0.05$ comparing low to moderate and c= $p \leq 0.05$ comparing low to high.

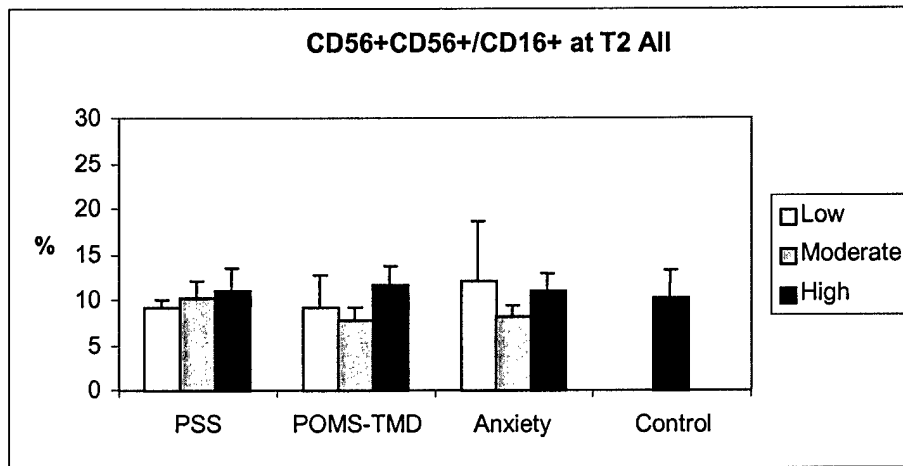


Figure 19. NK phenotypic analysis for biopsied women at T2 who had low, moderate, or high levels of either perceived stress, total mood disturbance, or anxiety, and in comparison to non-biopsied control women. Peripheral blood was collected and PBMC were assessed for the percentage of CD56/16+ & CD56+ by flow cytometry. N for; PSS for low= 6, moderate= 14, high= 8 . Control=8. POMS-TMD for low= 7, moderate= 14, high= 8. Anxiety for low= 3 , moderate= 18 , high = 10. Bars represent the mean values \pm S.E. No statistical differences were found.

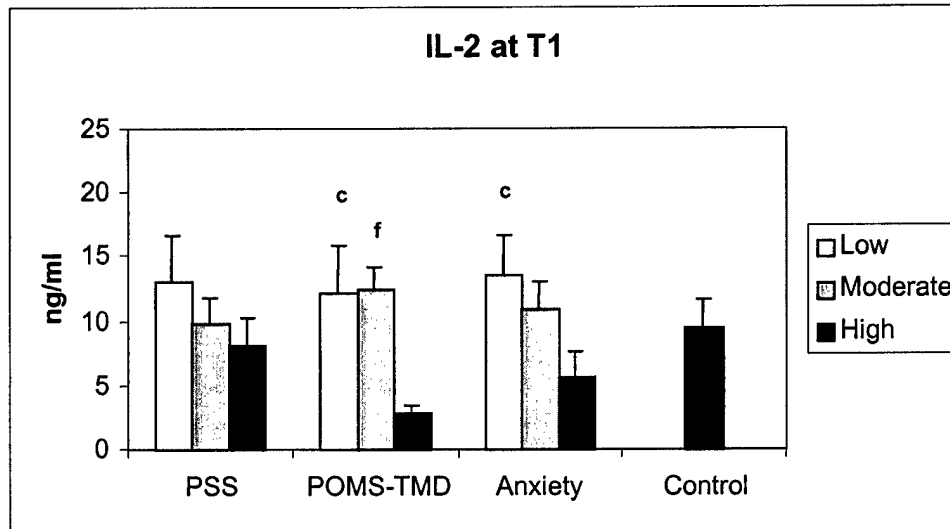


Figure 20. PBMC production of IL-2 is depicted for benign biopsied women at T1 who had low, moderate, or high levels of either perceived stress, total mood disturbance, or anxiety, and in comparison to non-biopsied control women. Peripheral blood was collected and PBMC were activated with PMA/PHA and culture supernatants were collected at 48 hr. Cytokine concentration was determined by ELISA. N for; PSS for low= 8, moderate= 24, high= 12 . Control=19. POMS-TMD for low= 8 , moderate= 25 , high= 11. Anxiety for low= 9 , moderate= 22 , high = 13. Bars represent the mean values \pm S.E. Statistical comparisons are as follows. Statistical comparison between low and high, $c=p \leq 0.05$. Statistical comparison between moderate and high; $f=p \leq 0.01$.

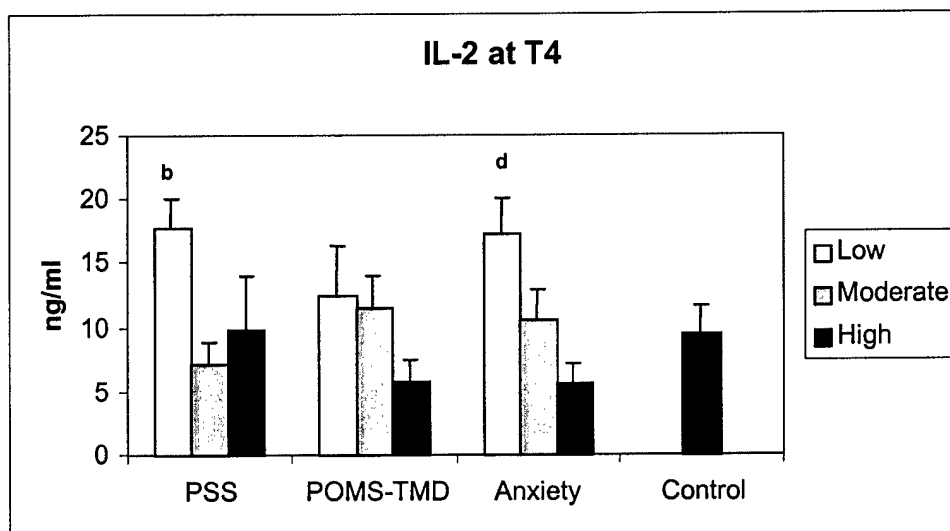


Figure 21. PBMC production of IL-2 is depicted for benign biopsied women at T4 who had low, moderate, or high levels of either perceived stress, total mood disturbance, or anxiety, and in comparison to non-biopsied control women. Peripheral blood was collected and PBMC were activated with PMA/PHA and culture supernatants were collected at 48 hr. Cytokine concentration was determined by ELISA. N for; PSS for low= 5, moderate= 19, high= 7. Control=19. POMS-TMD for low= 5, moderate= 15, high= 11. Anxiety for low= 4, moderate= 16, high = 10. Bars represent the mean values \pm S.E. Statistical comparisons for all figures are as follows. Statistical comparison between experimental means for low to moderate, $b=p \leq .01$. Statistical comparison between low and high, $d=p \leq .01$.

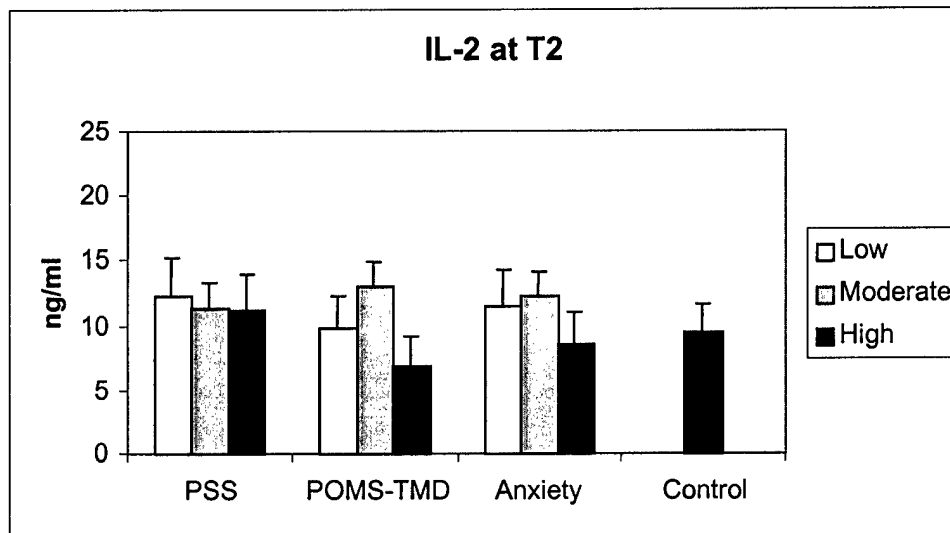


Figure 22.. PBMC production of IL-2 is depicted for benign biopsied women at T2 who had low, moderate, or high levels of either perceived stress, total mood disturbance, or anxiety, and in comparison to non-biopsied control women. Peripheral blood was collected and PBMC were activated with PMA/PHA and culture supernatants were collected at 48 hr. Cytokine concentration was determined by ELISA. N for; PSS for low= 8, moderate= 20, high= 13 . Control=19. POMS-TMD for low= 10 , moderate= 23 , high= 9 . Anxiety for low= 8 , moderate= 23 , high = 10. Bars represent the mean values \pm S.E. No statistical differences were found.

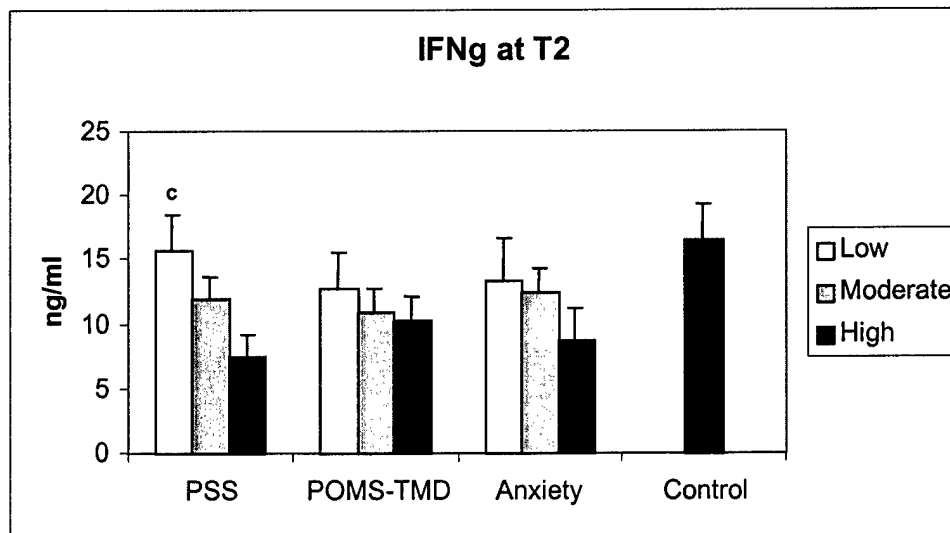


Figure 23. PBMC production of IFN γ is depicted for benign biopsied women at T2 who had low, moderate, or high levels of either perceived stress, total mood disturbance, or anxiety, and in comparison to non-biopsied control women. Peripheral blood was collected and PBMC were activated with PMA/PHA and culture supernatants were collected at 48 hr. Cytokine concentration was determined by ELISA. N for; PSS for low= 13, moderate= 27, high= 14 . Control=21. POMS-TMD for low= 16 , moderate= 27 , high= 11 . Anxiety for low= 13 , moderate= 28 , high = 13. Bars represent the mean values \pm S.E. No statistical differences were found.

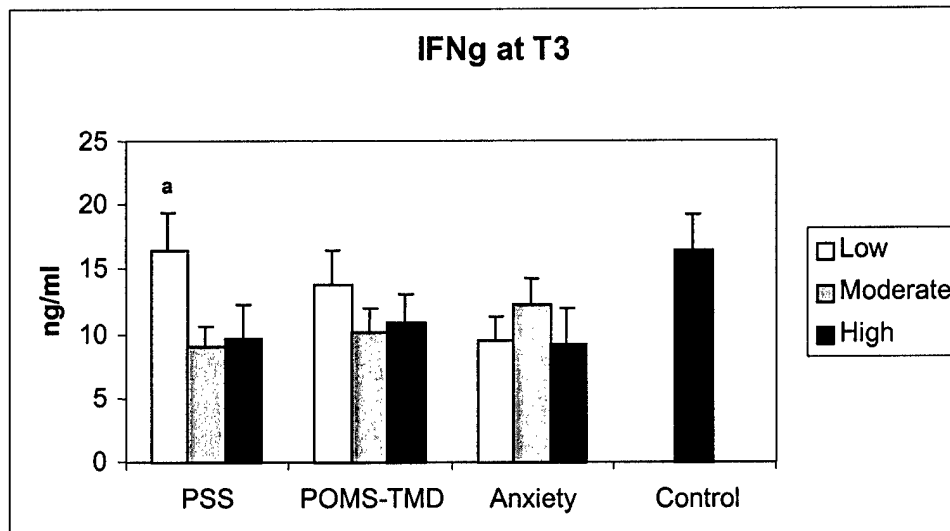


Figure 24. PBMC production of IFN γ is depicted for benign biopsied women at T3 who had low, moderate, or high levels of either perceived stress, total mood disturbance, or anxiety, and in comparison to non-biopsied control women. Peripheral blood was collected and PBMC were activated with PMA/PHA and culture supernatants were collected at 48 hr. Cytokine concentration was determined by ELISA. N for; PSS for low= 9, moderate= 22, high= 12 . Control=21. POMS-TMD for low= 9 , moderate= 22 , high= 10 . Anxiety for low= 9 , moderate= 23 , high = 10. Bars represent the mean values \pm S.E. Statistical comparisons for all figures are as follows. Statistical comparison between means for low to moderate, a=p \leq 0.05.

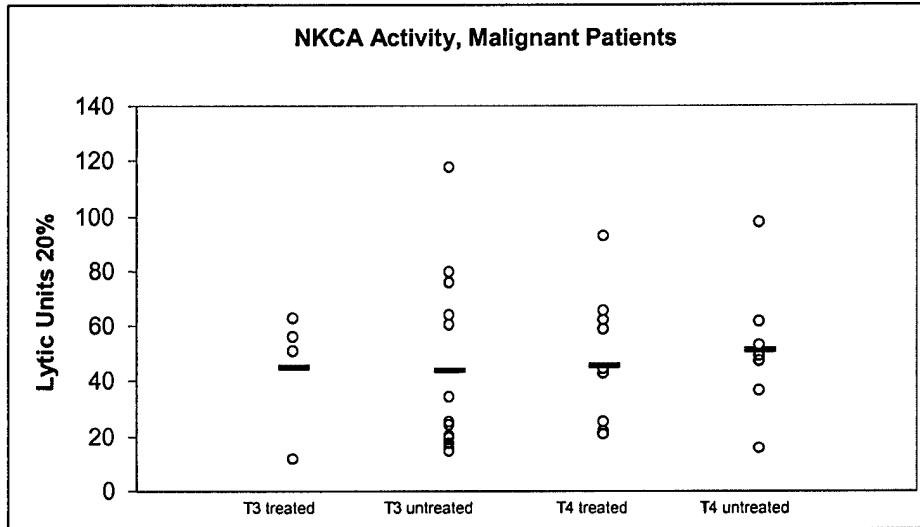


Figure 25. NKCA, expressed as lytic units at 20%, is compared for biopsied women with malignant findings who were either therapeutically treated or not at T3 and T4. Peripheral blood was collected post breast biopsy. NKCA was measured using K562 tumor cells as the target. N for; treated subjects at T3= 4, untreated subjects at T3= 13, treated subjects at T4= 10, untreated subjects at T4= 7. Solid lines represent mean values.

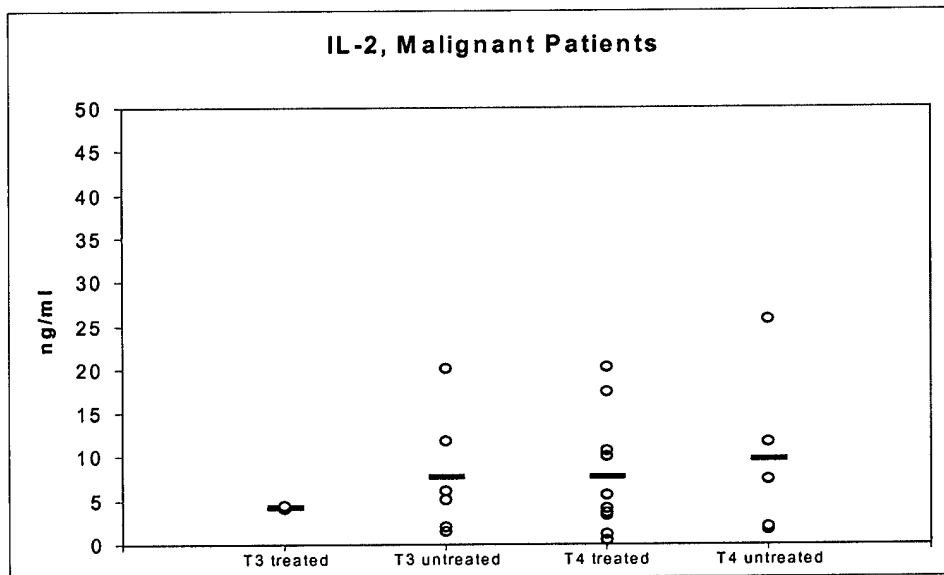


Figure 26. PBMC production of IL-2 is compared for biopsied women with malignant findings who were either therapeutically treated or not at T3 and T4. Peripheral blood was collected post breast biopsy. PBMC were activated with PMA/PHA and culture supernatants were collected at 48 hr. Cytokine concentration was determined by ELISA. N for; treated subjects at T3= 2, untreated subjects at T3= 6, treated subjects at T4= 10, untreated subjects at T4= 5. Solid lines represent mean values.

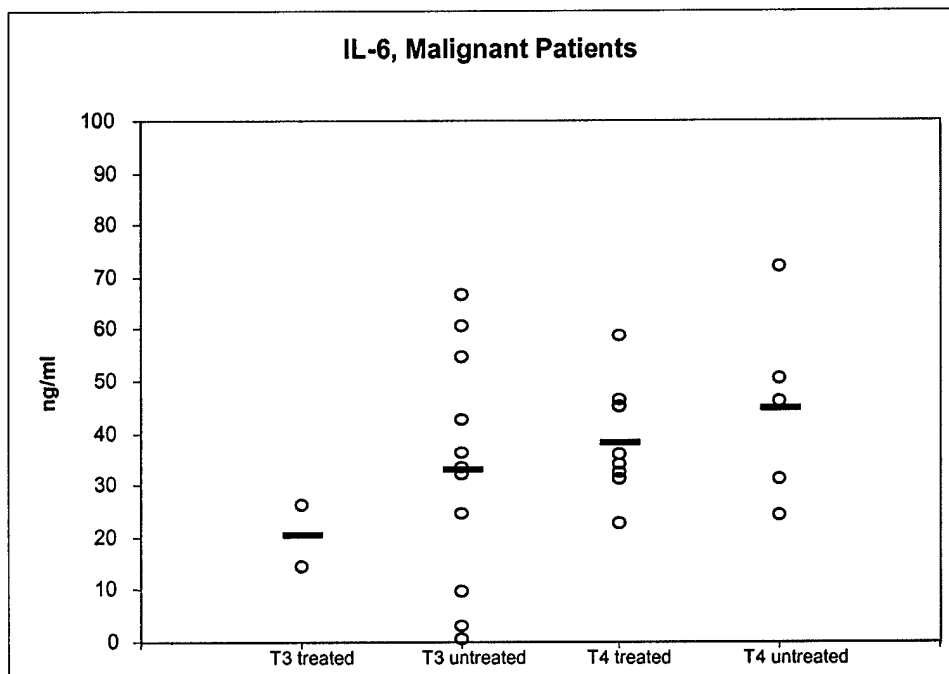


Figure 27. PBMC production of IL-6 is compared for biopsied women with malignant findings who were either therapeutically treated or not at T3 and T4. Peripheral blood was collected post breast biopsy. PBMC were activated with PMA/PHA and culture supernatants were collected at 48 hr. Cytokine concentration was determined by ELISA. N for; treated subjects at T3= 2, untreated subjects at T3= 11, treated subjects at T4= 8, untreated subjects at T4= 5. Solid lines represent mean values.

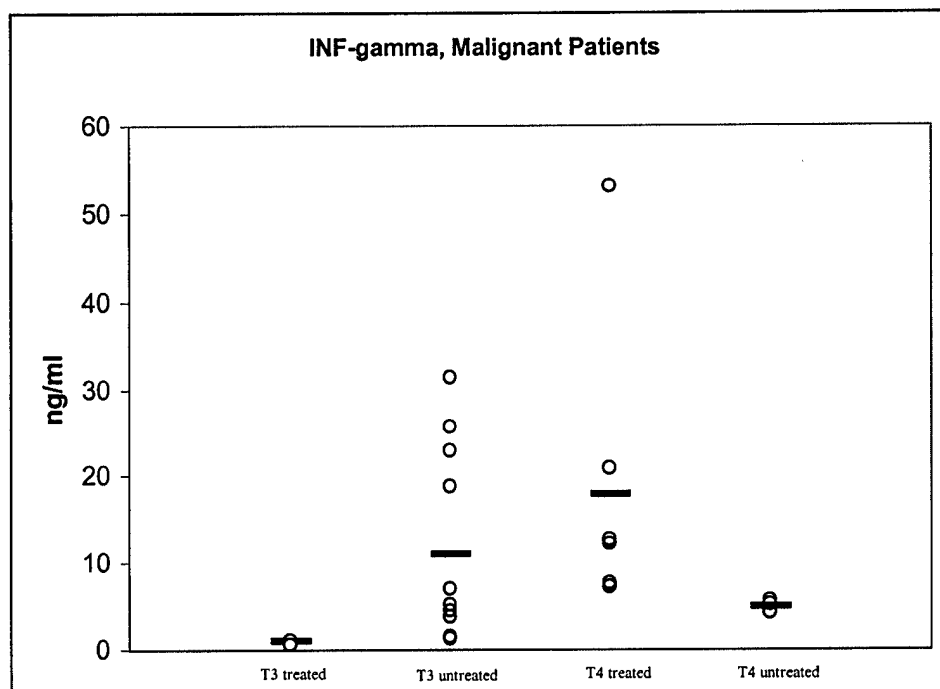


Figure 28. PBMC production of IFN γ is compared for biopsied women with malignant findings who were either therapeutically treated or not at T3 and T4. Peripheral blood was collected post breast biopsy. PBMC were activated with PMA/PHA and culture supernatants were collected at 48 hr. Cytokine concentration was determined by ELISA. N for; treated subjects at T3= 2, untreated subjects at T3= 11, treated subjects at T4= 7, untreated subjects at T4= 3. Solid lines represent mean values.

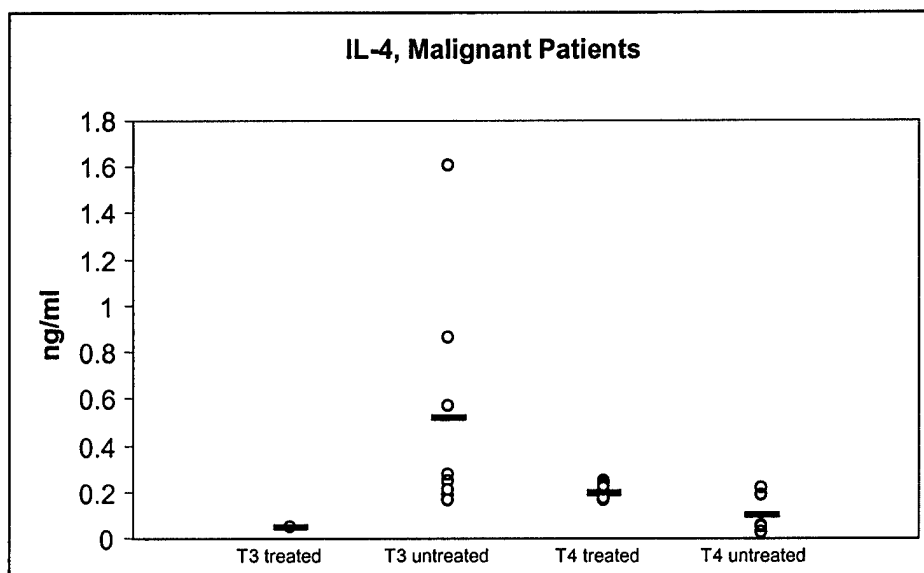


Figure 29. PBMC production of IL-4 is compared for biopsied women with malignant findings who were either therapeutically treated or not at T3 and T4. Peripheral blood was collected post breast biopsy. PBMC were activated with PMA/PHA and culture supernatants were collected at 48 hr. Cytokine concentration was determined by ELISA. N for; treated subjects at T3= 1, untreated subjects at T3= 8, treated subjects at T4= 10, untreated subjects at T4= 6. Solid lines represent mean values.

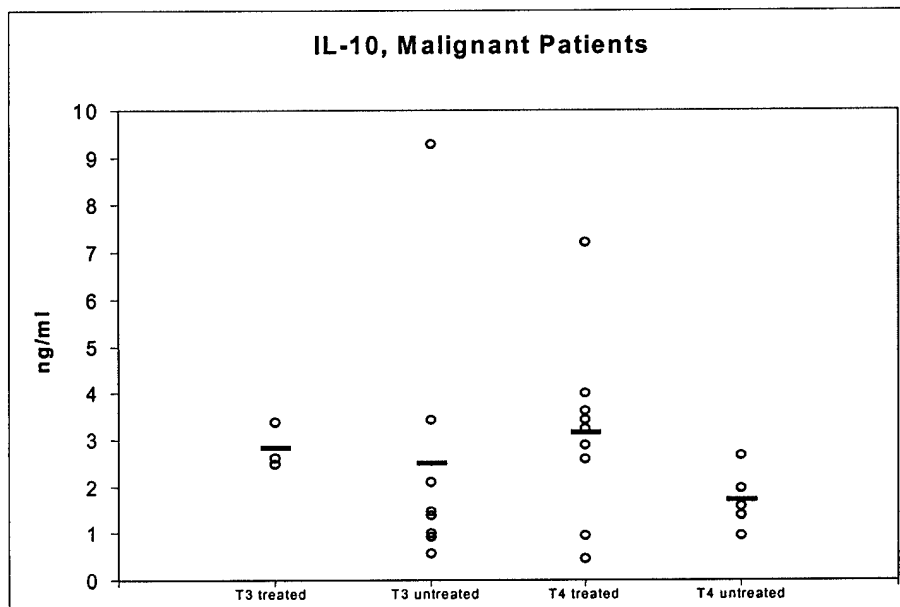


Figure 30. PBMC production of IL-10 is compared for biopsied women with malignant findings who were either therapeutically treated or not at T3 and T4. Peripheral blood was collected post breast biopsy. PBMC were activated with PMA/PHA and culture supernatants were collected at 48 hr. Cytokine concentration was determined by ELISA. N for; treated subjects at T3= 3, untreated subjects at T3= 8, treated subjects at T4= 9, untreated subjects at T4= 5. Solid lines represent mean values.

Table 1.**Numbers of Malignant Patients**

PSS T1			ANXIETY T1			POMS-TMD T1		
High	Moderate	Low	High	Moderate	Low	High	Moderate	Low
n=3	n=11	n=6	n=3	n=8	n=7	n=2	n=8	n=9
PSS T2			ANXIETY T2			POMS-TMD T2		
High	Moderate	Low	High	Moderate	Low	High	Moderate	Low
n=5	n=9	n=6	n=4	n=16	n=6	n=5	n=9	n=6
PSS T3			ANXIETY T3			POMS-TMD T3		
High	Moderate	Low	High	Moderate	Low	High	Moderate	Low
n=4	n=11	n=7	n=2	n=16	n=5	n=5	n=10	n=8
PSS T4			ANXIETY T4			POMS-TMD T4		
High	Moderate	Low	High	Moderate	Low	High	Moderate	Low
n=3	n=8	n=6	n=1	n=7	n=9	n=2	n=4	n=12

High, moderate, and low categories were based upon responses of all biopsied women at T1.

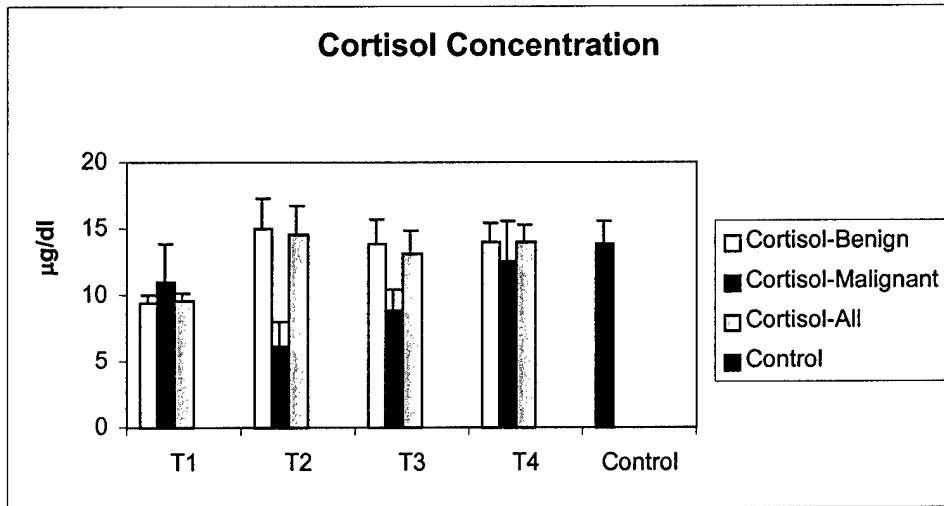


Figure 31. Cortisol concentrations depicted for all biopsied women and for women with benign findings, malignant findings, and in comparison to non-biopsied control women. Cortisol was measured by radioimmune assay. Cortisol: N for; Benign at T1=61 , Benign at T2=69 , Benign at T3=62 , Benign at T4=62 . Malignant at T1=7 , Malignant at T2=10 , Malignant at T3=10 , Malignant at T4=8 . Control=15. All at T1=68, All at T2=79, All at T3=72, All at T4=70. Bars represent the mean values \pm S.E.

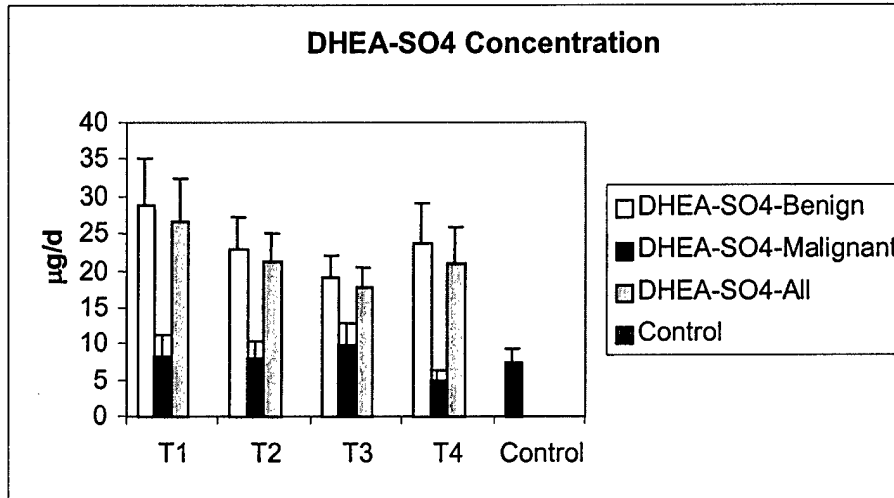


Figure 32. DHEASO4 concentrations depicted for all biopsied women and for women with benign findings, malignant findings, and in comparison to non-biopsied control women. DHEASO4 was measured by radioimmune assay. DHEASO4: N for; Benign at T1=60 , Benign at T2=69 , Benign at T3=59 , Benign at T4=57. Malignant at T1=7, Malignant at T2=10, Malignant at T3=10, Malignant at T4=10. Control=14. All at T1=67, All at T2=79, All at T3=69, All at T4=67. Bars represent the mean values \pm S.E.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
Follow the sample format on preceding page for each person. **DO NOT EXCEED FOUR PAGES.**

NAME		POSITION TITLE	
Linda Janusek		Professor, School of Nursing	
EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)			
INSTITUTION AND LOCATION	DEGREE (if applicable)	YEAR(s)	FIELD OF STUDY
Bradley University, Peoria, IL	B.S.	1974	Nursing
University of Illinois at Chicago	Ph.D.	1978	Physiology & Biophysics
Loyola University Chicago	Post Doc	1978-1980	Sepsis Pathophysiology

Professional Experience:

1978-1983 Assistant Professor, Maternal Child Health Nursing, Loyola University of Chicago, Maywood, IL
Adjunct Faculty, Dept. of Physiology, School of Medicine, Loyola Univ. of Chicago, Maywood, IL
1984 – 1977 Associate Professor, Maternal Child Health Nursing, Loyola University of Chicago, Maywood, IL
Adjunct Faculty, Dept. of Physiology, School of Medicine, Loyola Univ. of Chicago, Maywood, IL
1977 – Present Professor, Maternal Child Health Nursing, Loyola University of Chicago, Maywood, IL
Adjunct Faculty, Dept. of Physiology, School of Med., Loyola Univ. of Chicago, Maywood, IL

Relevant Training:

Molecular Mycology Course – Marine Biology Laboratory, Woods Hole, MA, Summer, 1998
Research Mentored Scientist Award – “Infant Antifungal Defense,” 1996-2000 (K01-NR-00085)

Professional Honors:

Sigma Theta Tau (Alpha Beta Chapter)
Phi Theta Kappa, National Honor Society, National Vice President
Young Investigator Award, Circulatory Shock Society, 1980
Alumni Award, Bradley University, 1990
Article of the Year Award, Dimensions of Critical Care Nursing, 1993

Federal Grant Review Boards:

Special Scientific Review Committee, Nursing/Biology Interface, NINR, Nat. Institutes Health (NIH), 1993
Ad hoc Study Section Member, Health Promotion and Disease Prevention (Nursing, Initial Review Group), NIH, 1994
Study Section Member, Health Promotion and Disease Prevention (Nursing Initial Review Group) NIH, 1995-1998
Speical ad hoc NIH Review Group, AIDS, ZRG1 AARR-5 O2 S, August, 2000

Relevant Publications:

1. Marotta, S.F., Witek-Janusek, L., Yu, M., Sithichoke, N. & Garcy, A.M. Adrenal and plasma corticosterone of hepatectomized rats: Responses during hepatic restoration. Hormone and Metabolic Research, 10:243-247, 1978.
2. Filkins, J.P., Witek-Janusek, L. & Yelich, M.R.. Role of insulin and insulin-like activity in the hypoglycemic response to endotoxin. In: Bacterial Endotoxins and Host Response, M. Agarwal, (Ed.), N. Holland: Elsevier (pp. 361-379), 1980.
3. Witek-Janusek, L. & Marotta, S.F. Status of the pituitary-adrenocortical-liver axis following partial hepatectomy. Proceeding Society Experimental Biology Medicine, 166:210-215, 1981.
4. Witek-Janusek, L. & Filkins, J.P. Relation of endoxin structure to hypoglycemic and insulin-like actions. Circulatory Shock, 11:23-34, 1983.
5. Witek-Janusek, L., Yu, M. & Marotta, S.F. Nycthemeral and stress response of the hypothalamic-pituitary adrenocortical system of partially hepatectomized rats. Aviation, Space and Environmental Medicine, 55: 538-541, 1984.
6. Yelich, M.R., Witek-Janusek, L. & Filkins, J.P. Glucose dyshomeostasis in endotoxemia: Direct versus monokine-mediated mechanisms of endotoxin action. In: The Immunobiology and Immunopharmacology of Bacterial Endotoxins. A Szentivanyi and H. Friedman (Eds.), New York: Plenum (pp. 111-132) 1985.

7. **Witek-Janusek, L.** Maternal ethanol ingestion: Effect on maternal and neonatal glucose balance. American Journal of Physiology, 251 (Endocrinology Metabolism 14): E178-E184, 1986.
8. **Witek-Janusek, L.** Pituitary-adrenal response to bacterial endotoxin in the developing rat. American Journal of Physiology, 255 (Endocrinology Metabolism)18:E525-E530, 1988.
9. **Witek-Janusek, L. & Yelich, M.R.** Neonatal sepsis: metabolic and hormonal considerations. In: Perspectives in Shock Research, R.F. Bond, I. Chaudry and R. Adams (Eds.) New York: Alan R. Liss, (pp.111-132) 1988.
10. **Witek-Janusek, L.** Metabolic alkalosis: Pathophysiology and the resulting signs and symptoms. Nursing, 20(6);52-53, 1990.
11. **Witek-Janusek, L.** Metabolic acidosis: Pathophysiology, signs and symptoms. Nursing, 20(7): 52-53, 1990.
12. **Witek-Janusek, L. & Ratmeyer, J.K.** Sepsis in the young rat: maternal milk protects during cecal ligation and puncture sepsis but not during endotoxemia. Circulatory Shock, 33:200-206, 1991.
13. Zeller, W. P., Goto, M., **Witek-Janusek, L.** & Hurley, R.M. Mortality, temporal substrate, and insulin responses to endotoxic shock in zero, ten and twenty-eight day old rats. Surg. Gynecol. Obstet., 173:375-383, 1991.
14. Klein, D.M. & **Witek-Janusek, L.** Advances in immunotherapy for sepsis. Dimensions of Critical Care Nursing, 11:75-89, 1992.
15. **Witek-Janusek, L.** Structure and function of the immune system. In: Medical Surgical Nursing: A psychophysiologic Approach (4th Ed.) J.M. Black, & E. Matassarin-Jacobs (Eds.), Luckmann and Sorenson's. (pp. 529-547), 1993.
16. Yelich, M.R. & **Witek-Janusek, L.** Glucose, lactate, insulin and somatostatin responses to endotoxin in developing rats. Shock, 2:438-444, 1994.
17. **Witek-Janusek, L. & Cusack, C.** Neonatal sepsis: Confronting the challenge. Critical Care Nurs Clinics N America, 6:405-419, 1994.
18. Letizia, M. & **Witek-Janusek, L.** Fever as a self-defense mechanism. MedSurg Nursing, 3:373-377, 1994.
19. **Witek-Janusek, L. & Yelich, M.R.** Role of the adrenal cortex and medulla in the young rats' glucoregulatory response to endotoxin. Shock, 3:434-439, 1995.
20. Werner, J. & **Witek-Janusek, L.** Stress. In Medical Surgical Nursing. Heikemper, M.M. & Lewis, S. (Eds.), pp 92-125, 1999.
21. **Witek-Janusek, L., Stoddard, J. and Mathews, H.L.** Trauma-induced immune dysfunction: A challenge for critical care. Dimensions of Critical Care Nurs, 17:187-199, 1998.
22. **Witek-Janusek, L., Cusack, C., and Mathews, H.L.** *Candida albicans*: An opportunistic threat to the critically ill low birth weight infant. Dimensions of Critical Care Nurs, 17:243-255, 1998.
23. Mathews, H.L. & **Witek-Janusek, L.** Antifungal activity of interleukin-2 activated natural killer (NK1.2) lymphocytes against *Candida albicans*. J. Medical Micro, 47:1-8, 1998.
24. Mathews, H.L., Conti, S., **Witek-Janusek, L.** & Polonelli, L. Effect of *Pichia anomala* killer toxin on *Candida albicans*. Medical Mycology, 36: 199-204, 1998.
25. Stover, A.G., **Witek-Janusek, L.** & Mathews, H.L. A method for flow cytometric analysis of *Candida albicans* DNA. J. Micro. Methods, 33:191-196, 1998.
26. Shareef, M.J., Myers, T.F., Mathews, H.L., & **Witek-Janusek, L.** Reduced capacity of neonatal lymphocytes to inhibit the growth of *Candida albicans*. Biology of Neonate. 75:31-39, 1999.
27. Robinson, P., Mathews, H.L. & **Witek-Janusek, L.** Stress and HIV Disease Progression: Psychoneuroimmunological framework. J. Assoc. Nurses Aids Care, 10:21-31, 1999.
28. **Witek-Janusek, L., & Mathews, H.L.** Differential effects of glucocorticoids on colony stimulating factors (CSFs) produced by neonatal mononuclear cells. Pediatric Research, 46; 1-6, 1999.
29. **Witek-Janusek, L. & Mathews, H.L.** Stress, Immunity and Health. In: Stress and Coping, Virginia Hill Rice (Ed.), Sage Publications, Thousand Oaks, pps. 47-67, 2000.
30. Robinson, P.F., Mathews, H.L. & **Witek-Janusek, L.** Stress reduction in HIV disease: A review of intervention studies using a psychoneuroimmunology framework. J. Assoc. Nurses Aids Care, 11: 56-65, 2000.
31. Nagabusham, M., Mathews, H.L. & **Witek-Janusek, L.** Aberrant nuclear expression of AP-1 and NFkB in lymphocytes of women stressed by the experience of breast biopsy. Brain Behavior Immunity, 15:78-84, 2001.
32. **Witek-Janusek, L. and Mathews, H.L.** Infant susceptibility to *Candida albicans*: Reduced lymphocyte mediated antifungal capacity. Submitted.
33. **Witek-Janusek, L. and Mathews, H.L.** Immunological and psychological analysis of women experiencing breast biopsy. Brain, Behav, Immunity, Submitted, 2001.
34. Mathews H. L. and **Witek-Janusek, L.** Host Defense Against Oral, Esophageal, and Gastrointestinal Candidiasis. In: Candida and Candidiasis. R. Calderone (Ed.), ASM Press. Washington, pp. 179-192, 2002.

Research Projects Ongoing or Completed During the Last 3 Years:

Title – “Infant Antifungal Defense”

Agency – National Institute of Nursing Research – K01 – NR-00085

Principal Investigator – Linda Witek Janusek

Period – 9-1-96 to 8-31-00

The overall objective of this project is to understand the antifungal capacity of lymphocytes and PMNs of infants within the context of clinical variables (birth weight, gestational age, and severity of illness). The ability of infant lymphocytes and PMNs to inhibit growth of *Candida albicans* and to adhere to the fungus is being assessed, as well as the production of cytokines in response to *C. albicans*.

Title – “Stress and Immunity: Implications for Breast Cancer”

Agency – National Cancer Institute, (R03- CA – 72848)

Principal Investigator – Linda Witek Janusek

Period – 10-1-97 to 11-30-98

This objective of this small grant was to determine the psychological, endocrine and immunological response in women pre and post breast biopsy. Psychological stress, anxiety, mood disturbances, and immune response were measured at two time points before and two time points after breast biopsy.

Title – “Psycho-Endocrine-Immune Profile: Implications for Quality of Life in Breast Cancer Patients”

Agency – Department of Defense Breast Cancer Research Fund (BC – 971316)

Principal Investigator – Linda Witek Janusek

Period – 11-1-98 to 10-31-01

The aim of this project is to understand the effect of psychological stress on immunological function. Stress, anxiety and mood disturbance and immunological function are being measured over 4 time periods in woman during breast cancer diagnosis. Indices of immune function include Natural Killer cell activity and cytokine production. It is hypothesized that stress and emotions can alter immune response toward tumor targets.

Title – “Psycho-Endocrine-Immune Response to Mindfulness-Based Meditation in HIV-Infected Individuals”

Agency – Catholic Health Partners

Co-Principal Investigator – Linda Witek Janusek

Period – 11/1/98 to 10/31/01

The aim of this project is to examine the effects of an eight week structured stress reduction program on perceived stress, mood, anxiety, natural killer cell activity and functional well being of HIV infected individuals. Subjects are studied longitudinally across 4 time periods.

CHAPTER 3

Stress, Immunity, and Health Outcomes

Linda Witek-Janusek and Herbert L. Mathews

The assumption that psychological stress, physical stress, mood, and behavior modulate the immune system, and predispose an individual to illness, is centuries old. In the sixteenth century, the Greek physician Galen observed that melancholy women were more predisposed to the development of tumors. Today, the assumption is widely held that stress, emotions, and behavior affect health, well-being, and predisposition to disease. For example, a character proclaims in Woody Allen's film *Manhattan*, "I can't express anger, I grow a tumor instead." Only recently, however, has this mind-immune relationship been subjected to rigorous scientific inquiry.

The organized establishment of the science of psychoneuroimmunology is often

credited to Robert Ader, who first introduced this term in his presidential address to the American Psychosomatic Society (Ader, 1980). Ader defined *psychoneuroimmunology* as the study of the interactions among behavioral, neural, endocrine (neuroendocrine), and immunological processes of adaptation. The central premise is that an individual's response and adaptation to the environment is an integrated process involving interactions among the nervous, endocrine, and immune systems. This is in contrast to the traditional view of the immune system in which it is autonomous and functions independently of the other organ systems of the body. Today, psychoneuroimmunology is a multidisciplinary science that includes nurses, psychologists, immunologists, microbiologists, neuro-

AUTHORS' NOTE: This chapter is dedicated to the memory of my (LWJ) mentor and my friend, Dr. Sabath F. Marotta (1929-1996), who introduced me and numerous other nurses to scientific inquiry and stress physiology. May his memory live on in our collective contributions to the field of stress. This work was supported in part by the Department of the Army (DAMD-98-8120), the National Cancer Institute (CA-77120-01), the National Institute of Nursing Research (NR-00085), the National Institute of Allergy and Infectious Disease (AI-31127), Catholic Health Partners, and the Cancer Federation. The expertise of Josh Takagishi and Maribel Barrigan is gratefully acknowledged. The content of this chapter does not reflect the position or the policy of the Department of the Army or the U.S. government.

scientists, endocrinologists, and others. The collective aims of these scientists are to explore and explain the common belief that one's behavior and emotions can influence stress, immunity, and health outcome.

Despite the recent development of psychoneuroimmunology as a discipline, initial evidence that linked stress to the immune system was reported by Hans Selye in the 1930s. In his general adaptation syndrome, Selye described a triad of responses to acute physical stress that consisted of adrenal gland enlargement, gastric erosion, and thymic involution (Selye, 1936, 1976). Since then, scientific evidence confirming biological links among the nervous, endocrine, and immune systems has accumulated. These links include direct innervation of lymphatic tissue by the central nervous system and a shared communication network in which cells of the nervous, endocrine, and immune systems use common molecules and receptors to reciprocally modulate biologic activity. Thoughts, emotions, and behavior are known to activate anatomical and biochemical pathways, and these pathways in turn modulate immune function (La Via & Workman, 1998). Such observations and demonstrations have permitted advocates of psychoneuroimmunology to suggest that biobehavioral interventions aimed at strengthening immunocompetence may be an important component of holistic health care (Kiecolt-Glaser & Glaser, 1992).

► NEURAL-IMMUNE INTERACTIONS

The connection between the brain and the immune system is through direct innervation of lymphoid tissue and through the release of products from the brain that bind to membrane receptors on immunologically competent cells. It is clear that primary and secondary lymphoid tissues are innervated with noradrenergic and peptidergic nerve fibers (Felten et al., 1987). The Felten's immunohistochemical studies provide direct evidence of the close association between presynaptic

sympathetic nerve endings and lymphocytes and macrophages (Felten, Felten, Carlson, Olschowka, & Livnat, 1985). Experimentally produced brain lesions of the hypothalamus, hippocampus, and cerebral cortex alter immune function, suggesting a neural-immune interactive network of connections. Those areas of the brain that exert immunomodulatory effects are areas concerned with emotions and with visceral, autonomic, and neuroendocrine regulation, thus establishing the "hardwiring" between neural centers that process emotions and immune cells. Further verification of a neural-immune network or axis was provided when lymphocytes and macrophages were shown to bear receptors for adrenergic substances (both α - and β -adrenergic receptors) and various neuropeptide hormones, including vasoactive intestinal polypeptide, somatostatin, calcitonin gene-related peptide, substance P, and opioids (Stevens-Felten & Bellinger, 1997). The presence of such receptors on immune cells provides a mechanism whereby the immune system can respond to biochemical signals from the brain. Activation of these receptors leads to functional changes in immune response (i.e., lymphocyte proliferation, cytotoxicity, antibody production, and cytokine secretion).

A pivotal step in firmly establishing that the brain and immune system interact was accomplished by psychologists who, using animal models, demonstrated that classical psychological (Pavlovian) conditioning could produce immunologic changes (Ader & Cohen, 1993). Such conditioning and its effect on the immune system have been demonstrated clinically. For example, research has documented the occurrence of anticipatory immunosuppression prior to the administration of chemotherapy (Bovbjerg et al., 1990; Fredrikson, Furst, Lekander, Rothstein, & Blomgren, 1993).

Investigators continue to unravel the intricate interplay among the nervous, endocrine, and immune systems. The associated immunologic changes that occur in response to neuroendocrine mediators, however, are

highly complex, and often the characterization of putative interactions has been measured only *in vitro*, in which one variable is manipulated. *In vivo*, however, immunologically competent cells respond to multiple stimuli, including numerous so-called molecules of emotion, within a microenvironment. Ultimately, the net immune response is an integration of these stimuli. The multiple levels and complexity of such immune modulation are remarkable, considering that numerous peptide and hormonal mediators can augment and diminish immune function (Wang, Fiscus, Yang, & Mathews, 1995; Witek-Janusek & Mathews, 1999a). It remains to be determined how these peptides and mediators fit within a homeostatic framework or are altered by environmental perturbation.

Adding additional complexity, it is well established that not only do nerves and secretory products from the brain influence immune function but also the converse is true. Immune activation can modulate central nervous system activity. Hugo Besedovsky and collaborators conducted seminal studies, which demonstrated that antigenic challenge of the immune system can produce an increase in neural firing within the medial hypothalamus (Besedovsky, Felix, & Haas, 1977). A peak immune response was associated with a decrease in norepinephrine turnover. Cytokines produced by antigen-activated lymphoid cells altered the turnover of norepinephrine (Besedovsky, del Rey, Prada, Burri, & Honegger, 1983).

It is now understood that alterations in cytokine secretion subsequent to immune activation mediate behavioral effects often associated with illness. For example, interleukin-1 (IL-1), IL-6, and tumor necrosis factor- α (TNF- α) mediate sickness behavior (the fatigue, lethargy, and decreased appetite associated with infectious illness) (Dantzer et al., 1998). Because they are large protein molecules, cytokines do not readily cross the blood-brain barrier. They are believed to signal the brain by entering neural structures that do not possess tight capillary endothelial barriers, such as the organum vasculosum

laminae terminalis and area postrema. In addition, recent evidence indicates that cytokines released in the periphery can activate sensory afferents, such as vagal afferents, and signal central nervous system (CNS) areas involved in immune-related behavioral responses (Watkins, Meier, & Goehler, 1995).

Collectively, this evidence supports the concept of a dynamic neuroendocrine-immune network whereby soluble products of immunologically competent cells affect the CNS following antigenic challenge. It is this conceptualization that led Blalock to liken the immune system to a sensory organ capable of informing the CNS of an antigenic challenge (Blalock & Smith, 1985).

Both psychological and physical stressors are known to activate neuroendocrine pathways that interact with the immune system (Chrousos, 1998). Stressor activation leads to increased secretion of neurosecretory hormones from the hypothalamus, such as corticotropin-releasing hormone (CRH). In turn, these hypothalamic hormones regulate secretion of pituitary hormones, such as adrenocorticotropin hormone (ACTH) and endorphins. Because there are shared hormonal receptors on cells of the immune and neuroendocrine systems, reciprocal interactions between these systems are possible (Reichlin, 1993; Weigent & Blalock, 1999). Neuroendocrine secretory products have immunomodulatory effects and alter leukocyte function (e.g., the immunologic effects of glucocorticoids, endorphins, ACTH, growth hormone, and prolactin). These effects include the regulation of cytokine secretion, antibody synthesis, natural killer cell (NK) activity, and lymphocyte proliferation (Weigent & Blalock, 1999). The complex interactions between the neuroendocrine and immune systems are believed to, in part, downregulate inflammatory responses and limit continuous proliferation of lymphoid cells or excessive production of immune cell products or both (Munck & Guyre, 1986).

Interestingly, neuroendocrine hormones can be produced by leukocytes, the most well studied of which is proopiomelanocortico-

tropin (POMC). POMC is a precursor molecule for the hormones ACTH and endorphin. Although the role of hormones produced by immune cells is under investigation, it is likely that they function by autocrine/paracrine mechanisms within the local lymphoid microenvironment (Weigent & Blalock, 1999). The finding that immune cells can produce hormones normally secreted from the anterior pituitary emphasizes the close relationship of the immune and endocrine systems. Finally, immune cell secretory products (e.g., cytokines) alter neuroendocrine cell secretion. For instance, cytokines have actions at both the hypothalamic and pituitary levels. Cytokines, such as IL-1, IL-2, IL-6, and TNF, activate the adrenal axis, whereas IL-1 and TNF inhibit the gonadal axis and TNF and interferon-gamma (IFN- γ) suppress the thyroid axis (Weigent & Blalock, 1999).

The bidirectional nature of the neuroendocrine and immune systems likely accounts for the effect of stress on the immune system (Figure 3.1). Regulatory hormones and neuropeptides once believed to be confined to the brain or endocrine system or both are now known to be mutually expressed by all three systems (nervous, endocrine, and immune), and as a result each system may be capable of modulating the function of the other.

In summary, during the past 15 years empirical evidence has emerged that supports the existence of a communication network linking the nervous, endocrine, and immune systems. Psychological stimuli modulate the immune response either through direct activation of neural pathways that terminate in lymphoid tissue or by activation of neuroendocrine circuits leading to the release of molecules that bind to immunologically competent cells. Conversely, the immune system recognizes noncognitive stimuli, such as bacteria, fungi, and viruses, resulting in the secretion of an array of cytokines that act on receptors of the neuroendocrine system. Collectively, cognitive and noncognitive stimuli form a network, which is the basis for behaviorally induced al-

terations in immune function (Weigent & Blalock, 1999). It is likely that this neuroendocrine-immune network mediates the effect of stress on the development or progression or both of immune-based disease.

► STRESS AND IMMUNITY

Stressful life events, and the subsequent emotional and behavioral responses to these events, are commonly believed to alter immunity. When external demands (i.e., stressors) exceed an individual's adaptive capabilities, a stress response ensues (Lazarus & Folkman, 1984). It is the subsequent neurological and endocrinological changes that are believed to produce stress-elicited immune alterations. Studies during the past decade provide convincing evidence that psychological stress can affect the immune system (e.g., lymphocyte proliferation, NK activity, antibody synthesis, and cytokine production). These studies have been accomplished with animal models and in human stress situations, including both experimentally produced stress and naturalistic paradigms for stress evaluation. This chapter focuses on the major human stress paradigms.

Early studies supporting the effects of stress on immunity were conducted by the research team of Glaser and Kiecolt-Glaser. These investigators conducted a series of stress studies in medical students that demonstrated the immunosuppressive effects of in-class examinations (Kiecolt-Glaser, Garner, Speicher, Penn, & Glaser, 1984). The results of these studies indicate that the stress that accompanies examinations leads to a wide range of immunosuppressive effects, including decreased NK cell activity (Glaser, Rice, & Speicher, 1986), lymphocyte proliferation (Glaser et al., 1987), IFN- γ production (Glaser et al., 1986), IL-2 production (Glaser et al., 1990), and latent viral activation as evidenced by increased antibody titer to the virus (Glaser et al., 1992).

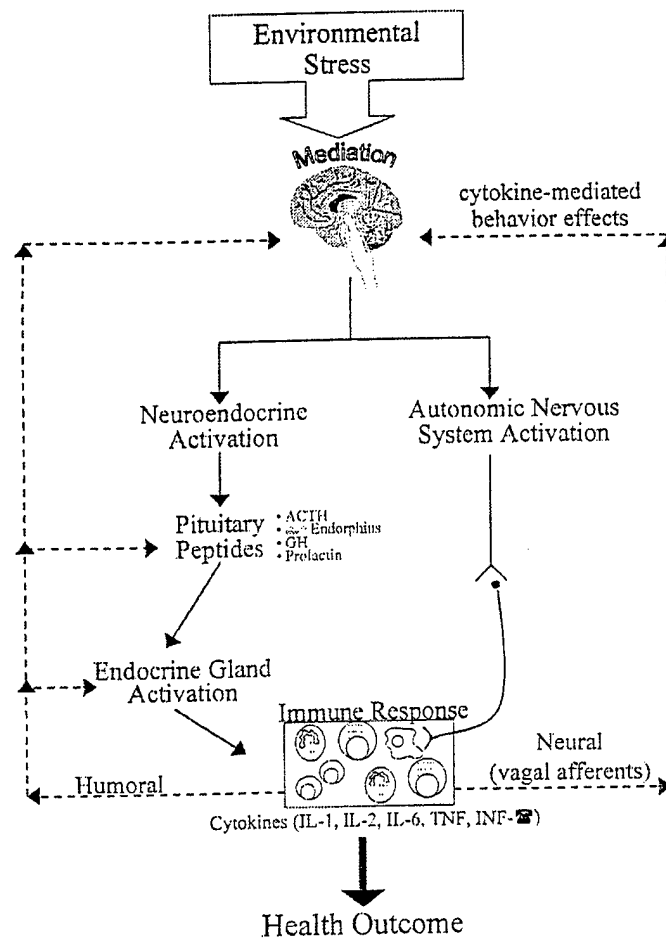


Figure 3.1. Summary and possible interconnections by which environmental stimuli, or stress, can affect the immune response and health outcomes. Perceived environmental stress is mediated by the central nervous system and can lead to neuroendocrine and autonomic nervous system activation. As a result, the immune response can be altered by autonomic nerve fibers that directly synapse with immune cells and by circulating catecholamines released from the adrenal medulla. In addition, further alteration can be produced by secretory products (hormones and neuropeptides) released from the pituitary and endocrine target glands (adrenal cortex, thyroid, ovaries, and testes). In turn, feedback (dashed lines) from immune cell products (cytokines) can modulate endocrine and central nervous system activity by either humoral or neural communication networks.

Furthermore, medical students with lower anxiety levels had faster and stronger immune responses to hepatitis B vaccination than did students with higher levels of anxiety (Glaser et al., 1992; Glaser, Kiecolt-Glaser, Malarkey, & Sheridan, 1998). Recently, examination stress was shown to alter cytokine

production that shifts the cytokine pattern away from a Th1 to a Th2 type of response (Maes et al., 1998). This shift is characterized by a decrease in secretion of IFN and an increased secretion of IL-10. The authors suggest that the shift in cytokine production may partially explain the increased incidence of

viral infection, latent viral expression, allergic and asthmatic reactions, and autoimmunity reported during times of high stress (Marshall et al., 1998).

The type of immune response seen as a result of stress is dependent on the acute versus chronic or repeated nature of the stressful event (McEwen, 1998). For example, acute stressors, such as parachute jumping, are correlated with a mobilization in the numbers of NK cells; this is likely attributable to a change in cell trafficking related to adrenergic arousal (Schedlowski et al., 1993) or glucocorticoid secretion (McEwen, 1998) or both. Studies such as these suggest that acute stress produces a redistribution of lymphocytes and macrophages in the body. These cells marginate on blood vessel walls and compartmentalize in the skin, lymph nodes, and bone marrow. It is theorized that acute stress activates the immune response and prepares the organism for potential encounters with an immunologic challenge. This activation may exacerbate autoimmune or allergic responses (Dhabhar, Miller, McEwen, & Spencer, 1996). Repeated or chronic stress, however, suppresses immune responsiveness, particularly cell-mediated immunity, and increases susceptibility to infectious challenge and tumor cells (McEwen, 1998). Chronic stressors, such as bereavement, caregiving, marital conflict, and divorce, impair the ability of NK cells to be lytic and to respond to cytokines (IFN- γ or IL-2) *in vitro* (Esterling, Kiecolt-Glaser, & Glaser, 1996; Herbert & Cohen, 1993; Kiecolt-Glaser, Dura, Speicher, Trask, & Glaser, 1991; Kiecolt-Glaser, Glaser, Cacioppo, & Malarkey, 1998). Other aspects of cellular immunity are also affected by chronic stress (Herbert & Cohen, 1993), including decreased lymphoproliferation, NK cell activity, numbers of circulating lymphocytes, as well as salivary and serum immunoglobulin levels.

The impact of chronic stress has been poignantly illustrated by assessing the immune response of individuals caring for relatives with Alzheimer's disease. Kiecolt-Glaser et al. (1987) found that such caregiving was accompanied by greater distress and

heightened levels of herpes virus-specific antibody (suggesting viral reactivation). Furthermore, elderly individuals experiencing the chronic stress of caring for a spouse with Alzheimer's disease had attenuated responses to the influenza vaccine and more physician-confirmed respiratory infections than control subjects. Health behaviors did not differ between the two groups.

Conversely, Irwin et al. (1991) reported no differences in NK cell activity between caregivers and control subjects. Esterling et al. (1996), however, found that both caregivers and former caregivers (those whose relative had died at least 2 years previously) had blunted NK cell activity compared to nonstressed control subjects. Interestingly, the results of this study suggested that the psychological and immunological aftermath of caregiving persists beyond the actual stressful experience. In an attempt to reverse the immunosuppressive effects of stress in the elderly, these investigators enrolled subjects in a 1-month stress-reduction program that used progressive muscle relaxation. This form of stress reduction produced a 30% increase in NK cell activity (Kiecolt-Glaser et al., 1985).

The type and magnitude of stress-elicited effects on the immune system are influenced by many factors. Such factors may relate to the stressor, such as the type, intensity, and duration of the stressful stimulus. The sampling time frame between the stressor and the immune response can also influence whether an effect can be measured. Furthermore, not all components of the immune system may respond to a stressor. Therefore, it is important that the immune parameter to be measured be carefully chosen within the context of the population or illness studied or both. A variety of host or subject factors will also influence the immune response to stress, such as age, preexisting illness, nutritional status, substance abuse, exercise habits, adequacy of sleep, coping, and social support (Kiecolt-Glaser & Glaser, 1988; Zeller, McCain, McCann, Swanson, & Colletti, 1996).

The primary criticism of many stress-immune studies is that although the immune change observed is often statistically signifi-

cant, the magnitude of the change is small and often within normal limits. Whether or not such a change in immune function is significant to health outcomes remains to be determined. There are studies, however, that have found that stress-induced immune changes can increase susceptibility to infectious disease and may also influence the course of disease (Cohen, Tyrel, & Smith, 1991; Spiegel, Bloom, Kraemer, & Gottheil, 1989). Such studies support the contention that even small changes in immune function may have health-related significance.

➤ STRESS-IMMUNITY AND HEALTH OUTCOMES

One fundamental question that remains unanswered in the field of psychoneuroimmunology is whether or not stress-induced alteration in immune function plays a role in disease development or disease progression or both. Numerous studies, although inconclusive, have shown stress to influence the course or progression of illness or disease (e.g., cancer, infectious disease, and HIV). Few studies, however, provide definitive evidence that links stress, immunity, and health outcomes. This area remains a challenge for researchers in psychoneuroimmunology.

One of a handful of well-controlled studies that examined the effect of psychological stress on susceptibility to illness was conducted by Cohen et al. (1991). They investigated the relationship between stress and the common cold using a viral challenge paradigm. Following extensive health and psychological assessment (for the previous 12 months), 394 volunteers were randomized to receive either a low infectious dose of a respiratory virus or saline. For 2 days prior to viral challenge and 7 days postchallenge, volunteers were quarantined. Rhinovirus infection was based on the development of clinical symptoms of a cold, the development of virus-specific antibodies, and the culture and isolation of the inoculated virus. The results revealed that psychological stress predicted susceptibility to colds among the initially healthy

people exposed to the respiratory virus. Psychological stress was operationalized as an index of the number of negative life events, the perceived impact of these negative life events, perceived stress, and negative affect. In a related study, Cohen et al. analyzed the relationship of an individual's social contacts to the development of the common cold. In 276 volunteers exposed to rhinovirus, a greater resistance to upper respiratory infection was exhibited in subjects who had the greatest diversity of social contacts (friends, family, and community). Interestingly, greater resistance to infection was related to increased numbers of social contacts and not to the absolute number of individuals involved in the social contacts (Cohen, Doyle, Skoner, Rabin, & Gwaltney, 1997). Recently, these investigators reported that acute stressful life events (less than 1 month in duration) were not associated with the onset of colds. Severe chronic stressors (1 month or longer in duration), however, were associated with the risk of cold development. The most prevalent chronic stressors for this study group were under- or unemployment or enduring interpersonal difficulties with family or friends (Cohen et al., 1998).

➤ STRESS AND WOUND HEALING

Studies of the effects of stress on wound healing and tissue repair have suggested that stress-induced neuroendocrine activation impairs healing and delays recovery. Both animal and human models of wound healing have been used to examine the effects of stress. In one study (Padgett, Marucha, & Sheridan, 1998), the effects of restraint stress on the healing of a sterile punch wound in rats were studied. Rats were subjected to restraint stress for 3 days prior to and for 5 days after wounding. Wound healing was measured using photography and image analysis. Compared to control rats, which were wounded but not restrained, healing was delayed an average of 3 days in the restraint stressed group. Treatment of the restraint stressed group with a glucocorticoid receptor

antagonist produced healing rates that were similar to those of control animals. These results demonstrate that restraint stress delayed wound healing. Because the glucocorticoid antagonist reversed this effect, the delay was likely due to a stress-induced increase in glucocorticoids. Padgett et al. (1998) hypothesized that the stress-induced elevation in glucocorticoids prevented the early part of wound healing in which macrophages move into the area to remove cellular debris and secrete growth factors, cytokines, and chemotactic factors needed for tissue repair. Glucocorticoids are well-known to suppress the inflammatory response, including the production of IL-1, IL-6, and TNF- α (Bendrup, Hilton, Meager, & Hamilton, 1993). The results of this study provide compelling evidence, albeit in an animal model, that disruption of neuroendocrine homeostasis by a stressor modulates wound healing.

The ability of stress to delay wound healing has also been shown in human stress paradigms. Kiecolt-Glaser, Marucha, Malarkey, Mercado, and Glaser (1995) studied the effects of chronic stress on caregivers (spouses) for patients suffering from Alzheimer's disease. Punch biopsy wounds were applied to caregivers and age-matched control subjects. The results indicated that wound healing was markedly delayed in the caregivers compared to control subjects. These differences were not related to other covariates, such as nutrition, sleep, or the presence of other illnesses. In another study, wound healing was delayed by the more acute and benign stress of academic examinations. Two punch biopsy wounds were placed on the hard palate of dental students during summer vacation and then on the contralateral side 3 days prior to the first major exam of the term. Mucosal wound healing took 3 days longer to complete during the exam period. The production of mRNA for IL-1 β was also reduced during the stress of examination (Marucha, Kiecolt-Glaser, & Favagehi, 1998).

In summary, the previously discussed studies provide compelling evidence suggesting that stress can impair tissue repair and

wound healing. This can have significant implications for recovery from injury and surgery, especially in vulnerable populations, such as individuals with diabetes, impaired tissue perfusion, and advanced age. Delayed healing of wounds increases the risk for wound complication by infectious pathogens, which can further prolong recovery and length of hospital stay. Nurses are in a pivotal position to recognize and reduce stress and to teach stress management skills. This has the potential to promote both healing and recovery and enhance health outcomes.

► STRESS-IMMUNITY AND CANCER

The immune system is believed to play a role in surveillance against malignantly transformed cells. It has been hypothesized that stress-induced suppression of immune cell activity (e.g., NK cell activity) may alter the clinical course of cancer. The relationship between NK cell activity and cancer is complex (Rosenberg & Lotze, 1986). NK cells, however, are also important in the control of viral infections (Trinchieri, 1989; Whiteside & Herberman, 1989). As such, NK cells may prevent the development of infectious complications in cancer patients who are often immunosuppressed.

There are a few highly intriguing studies that have examined the relationship between stress and immunity in cancer patients. Levy et al. (1990) found that estrogen receptor status predicted NK cell activity in 66 women with Stage I or II breast cancer 3 months after surgery with or without adjuvant therapy. These researchers also showed that social support contributed significantly to a regression model predicting higher NK cell activity. That is, the greater an individual's social support, the higher the individual's NK cell activity.

Andersen et al. (1998) studied stress-immune parameters in 116 women who were diagnosed with invasive breast cancer (Stages II and III). Women were enrolled within 4 months of their breast surgery but prior to

adjuvant therapy initiation. Stress was measured using the Impact of Event Scale, which is a self-report measure of intrusive and avoidance thoughts and behaviors (Horowitz, Wilner, & William, 1979). Using hierarchical multiple regression analysis, their results revealed that higher stress levels significantly predicted lower NK cell activity, diminished NK cell response to IFN- γ , and decreased lymphocyte proliferation (Andersen et al., 1998). It is noteworthy that this study controlled for extraneous variables that might also affect immunity, including age, stage of disease, nutritional status, and days since surgery. The results are intriguing and suggest that stress may play a pivotal role in women with cancer, possibly resulting in more susceptibility to cancer progression or infectious complications or both.

Researchers have begun to address the definitive question as to whether psychosocial interventions can produce health effects that slow cancer progression and promote survival (Fawzy, Fawzy, Arndt, & Pasnau, 1995; Greer & Brady, 1988; Spiegel, 1996). Randomized prospective trials have shown protective effects of psychosocial interventions on cancer progression (Spiegel, Sephton, Terr, & Stites, 1998). Fawzy et al. (1993) studied the effects of a behavioral intervention in patients with malignant melanoma. Subjects were randomized to an intervention consisting of six 90-minute sessions including health education, stress management, coping skills, and group discussion. Six months later, the intervention group showed reduced psychological distress and enhanced immune function (increased IFN- α and augmented NK cell activity) compared to the nonintervention group. Although no association between survival and NK cell activity was found, individuals with higher baseline NK cell activity had a decreased incidence of disease recurrence.

In another study, the effects of a home visit and educational intervention program for lymphoma and leukemia patients were investigated (Richardson, Shelton, Krailo, & Levine, 1990). The results showed that patients in the intervention group were more compli-

ant with their medical treatment. More important, when controlling for this difference, members of the intervention group lived significantly longer than members of the control group.

A landmark study that assessed the effect of behavioral intervention on cancer survival was conducted by David Spiegel and colleagues (1989). They reported compelling results suggesting that an intervention, characterized as supportive-expressive group therapy, increased the survival of women with advanced breast cancer. Fifty of 86 women with advanced breast cancer were randomly assigned to support groups. The groups were designed to build strong supportive bonds, encourage "emotional expressiveness" about cancer, confront fears of dying and death, reorder life's priorities, improve relationships with family and friends, enhance communication with and development of shared problem solving with physicians, and teach self-hypnosis for pain control (Spiegel et al., 1998). The women were followed for 10 years, and a significant 18-month increase in survival for women in the intervention group was observed. Further analysis of the results of this study, in which medical records were reviewed, showed no difference in therapeutic treatment that could account for the differences in survival. Rather, a correlation was found between group support and survival. Spiegel's research team is currently replicating this study with a larger group in which endocrine markers of stress and cellular immune response, including NK cell activity, are being measured in addition to survival. It is hypothesized that psychosocial support will buffer the immunological consequences of cancer-associated stress and thereby improve disease outcomes (Spiegel et al., 1998).

In addition to the ongoing study of Spiegel, Andersen and colleagues (1998) are conducting a prospective, randomized study evaluating the effectiveness of stress-reduction interventions on psychological, immunological, and survival outcomes in women with advanced breast cancer. The structured intervention includes several stress-reduction strat-

egies, such as progressive muscle relaxation, social and emotional interventions designed to increase the quality of life, and healthy living habits. The intervention is provided weekly for the first 4 months and monthly for an additional 8 months. Psychological and immunological variables are being measured, with survival being the ultimate end point of this ongoing longitudinal study (McNeil, 1998; Voelker, 1997).

The role of psychological stress in cancer progression or response to treatment or both remains controversial, as was expressed in an editorial by Cohen and Rabin (1998). They contend that it is not clear if the effects of behavioral interventions are due to an individual's greater adherence to a healthy lifestyle or to the behavioral intervention therapy or both. The results of behavioral-based intervention studies are highly provocative and difficult to ignore, however. Indeed, the results of the ongoing clinical trials will provide further data that will aid in the understanding of the importance of stress, its impact on the immune system, and cancer control.

► STRESS-IMMUNITY AND HIV

Individuals living with HIV face numerous stressors, such as family discord, change in occupation, economic hardship, social isolation, and bereavement (McCain & Zeller, 1996; Robinson, Mathews, & Witek-Janusek, 2000). Because the immune system plays a dominant role in the prevention of viral infections and in the suppression of latent viral infections, stress-induced changes in immune function may alter disease progression. Evidence suggests that stress-induced modulation of immunity may alter the course of HIV infection (McCain & Zeller, 1996; Robinson et al., 2000). Psychological variables are hypothesized to mediate host resistance to the HIV virus by modifying behavioral practices and by promoting an optimal neuroendocrine and immune milieu. Overall, most of these

studies are fraught with methodological difficulties, such as small and nonhomogeneous samples, lack of control for treatment and disease stage variables, inability to document or measure the presence of psychosocial stress in the sample, and lack of sensitive and relevant indices of immune measures. Nevertheless, the results are intriguing.

Goodkin, Fuchs, Feaster, Leeka, and Rishel (1992) studied stress-immune correlates in asymptomatic HIV-positive males. Although the sample size was small, the results suggested that men with a lower ability to cope with stress had lower total lymphocyte counts, whereas men with higher coping abilities had greater numbers of CD4+ T lymphocytes. A series of stress-immune studies have originated from the University of Miami's Center for the Biopsychosocial Study of AIDS; some of these studies have evaluated the psychoimmune effects of the stress of HIV antibody testing (i.e., test notification stress). This research team reported a significant relationship between increased anxiety (State Trait Anxiety Index [STAI]) at the time of notification of test results and decreased NK cell activity. No association with lymphocyte proliferation was found (Ironson et al., 1990).

In a similar study, during a 5-week period before and after HIV testing, seropositive subjects reported higher anxiety (STAI), higher depression, increased intrusive thoughts, and lower lymphocyte proliferation rates than seronegative subjects. Although plasma cortisol levels declined significantly in the seropositive group during the study period, they were within normal limits (Antoni et al., 1991). McCain and Cella (1995) found a significant relationship between high stress and lower CD4+ cell numbers in their study of a heterogeneous group (heterosexuals, minorities, injecting drug users, and those with various stages of disease progression) of 53 men with HIV disease. These same investigators examined the effect of a stress management intervention in HIV-positive individuals. Although a reduction in stress was demonstrated, they failed to show any significant ac-

companying change in immune function (McCain, Zeller, Cella, Urbanski, & Novak, 1996). In another intervention study, however, Esterling and colleagues (1992) measured antibody titers to Epstein-Barr virus (EBV) as the immune end point. Both HIV-positive and HIV-negative men in the 10-week program had significant decreases in anti-EBV viral encapsulated antigen when compared to their matched controls (Esterling et al., 1992). Because of the intriguing nature of these intervention studies, similar lines of research will likely be pursued in the future.

Although there is no clear mechanism for how stress influences HIV disease progression, Clerici and colleagues (1994) proposed an "immunoendocrinological" hypothesis implicating the potential role of elevated cortisol in the progression of HIV disease through modulatory effects on viral replication, cytokine modulation, and increased induction of apoptosis. Supportive evidence for this theory has been provided by reports that cortisol enhances HIV viral infections when added to cell culture medium containing human lymphocytes (Markham, Salahuddin, Veren, Orndorff, & Gallo, 1986) and HIV viral replication when added to monocyte cultures (Swanson, Zeller, & Spear, 1998). Norepinephrine, a major catecholamine released during stress, also accelerates HIV replication (Cole, Korin, Fahe, & Zack, 1998).

It is likely that studies examining psychoneuroimmune parameters in HIV disease are limited by the immune outcome variables measured. It is possible that psychological effects may not have a measurable impact on indices of HIV disease development or progression or both. More important, stress may play an important role in the HIV-infected person's susceptibility to opportunistic infection. Consequently, there is a need to design and implement studies aimed at determining the role of psychological stress on immune system indices designed to measure defense mechanisms important in host defense against opportunistic infection (Robinson et al., 2000). The nature of the stress-immune relationship in HIV

disease needs to be carefully evaluated within the context of currently used antiretroviral therapy and within the context of future therapeutic approaches. Such therapies may not only alter immune responsiveness in those with HIV but also influence the type of stress they encounter as they live with HIV.

➤ STRESS-IMMUNITY AND INFECTION

Vulnerable populations, such as cancer patients and persons with HIV, face a multitude of stressors. These stressors can influence the immune system and increase susceptibility to infectious diseases. Psychological stress seems to alter the susceptibility of individuals to infectious agents and influences the onset, course, and outcome of the pathology associated with infection (Biondi & Zannino, 1997). Moreover, infectious disease can be a stressor. The human body's response to infection and to immunological challenge resembles both physical and psychological stress (Dunn, Powell, Meitin, & Small, 1989).

Infection can activate the hypothalamic-pituitary-adrenal axis (HPA) axis and increase the synaptic release of norepinephrine and serotonin in the brain (Dunn, 1993). Thus, by physiological criteria, infection can be regarded as stressful. The activation of the HPA axis associated with immune responses has been interpreted as a signal to the brain indicating the presence of an infectious threat from the external environment, triggering a stress response (Blalock & Smith, 1985). Once an effective immune response has been initiated, the HPA axis is thought to negatively regulate the immune system by the release of glucocorticoids that limit the inflammatory response and prevent overreactivity and autoimmune phenomena (Besedovsky, del Rey, Sorkin, & Dinarello, 1986; Munck & Guyre, 1986). Thus, the effects of stress on the immune system and the effects of the immune system on the neurologic response to infection are a complex and interrelated series of

physiologic events with many reciprocal interactions. Many specific infectious states appear to have a clear association with stress.

Tuberculosis

Stress has long been associated with the pathogenesis of tuberculosis. With the recent resurgence of tuberculosis, understanding the potential role of stress in susceptibility to and progression of this infectious disease has become even more important. In previous studies, high rates of tuberculosis have been reported among socially isolated individuals and in schoolchildren and their teachers during periods of emotional stress, such as during war (Guyre, Girard, Morganelli, & Manganiello, 1988; Ishigami, 1919). These studies showed a reduced capacity of the infected individuals to phagocytize the infectious agent and suggested that stressful situations might serve as cofactors in the development of tuberculosis. Until recently, very little evidence existed to support this suggestion. Work using experimental animals has shown that HPA axis activation, induced by restraint stress, increased the growth of the tubercle bacillus (Zwilling et al., 1990). Adrenalectomy and treatment with the glucocorticoid receptor antagonist RU486 abrogated this effect. Furthermore, HPA axis activation suppressed phagocyte function and decreased the capacity of the animals to produce immune augmenting cytokines in response to the mycobacteria (Brown, Sheridan, Pearl, & Zwilling, 1993).

The effects of stress in experimental animals may have important implications in human disease. In an extensive study, tubercular patients were shown to have a dramatic increase in the number of stressful life events approximately 2 years prior to their hospitalization (Homes, Hawkins, Bowerman, Clarke, & Joffe, 1957). Likewise, mortality due to tuberculosis has been shown to be higher in subjects who have experienced divorce (Somers, 1979). It is possible that the reactivation of tuberculosis may be a consequence of suscepti-

ble populations being affected by stressors. Stress, mediated by neuroendocrine-immune interactions, may significantly contribute to this infectious disease, which continues to be a serious health hazard worldwide.

Viral Infections

Colds and influenza have been useful models to evaluate the role of psychoneuro-immunology in human disease. As discussed previously, Cohen et al. (1991) evaluated the significance of psychosocial factors on the common cold. Subjects were inoculated with respiratory viruses, and the risk of developing the infectious disease was directly associated with chronic stress. This study and many others showed similar effects of stress on the development of colds and influenza but no direct effect of stress on the immune system of the more susceptible individuals (Clover, Abell, Becker, Crawford, & Ramsey, 1989).

Many other studies have evaluated the effects of stress on latent viral infections caused by herpes simplex virus, EBV, and HIV. These viruses are typically latent in humans, and the hypothesis that stress favors viral reactivation has been evaluated. These studies have shown that exposure to acute psychological stressors (e.g., examinations and spousal discord) and chronic psychological stressors (e.g., nuclear disaster and caregiving) is associated with high antibody titers to these viruses. These viruses are thought to be controlled by normal host cell-mediated immune response. When stress reduces the cell-mediated immune response, the virus replicates and stimulates an antibody response that is typically non-protective (Kiecolt-Glaser et al., 1991). In the case of genital herpes, Kemeny, Cohen, Zegans, and Conant (1989) showed that a negative mood state was correlated with a decrease in CD8+ lymphocytes (the principal effector against herpesvirus) and herpetic lesion recurrence. Likewise, psychological stress has been shown to predispose an individual to the onset of infectious mononucleosis (Glaser et al., 1991). This work remains

controversial, however, and the role of psychoneuroimmunology in latent viral infections, viral reactivation, and immune stimulation requires further investigation.

Fungal Infections

Although the association between emotional stress and infectious mycological disease has been long suspected, only recently has considerable attention been paid to this association. Fungal infections are well-known to be associated with the stressful conditions of pregnancy, surgical trauma, cancer, organ transplants, long-term antibiotic use, corticosteroid therapy, diabetes mellitus, critical illness, and prematurity (Reszel, Mishra, Mishra, & Pierson, 1993; Shareef, Myers, Nagabhushan, Mathews, & Witek-Janusek, 1998; Witek-Janusek, Cusack, & Mathews, 1998).

Stress hormones such as cortisol and adrenaline are known to enhance pathogenesis of experimental fungal disease (Odds, 1988). For example, *Candida albicans* and related fungi are endogenous opportunists, and infections with these fungi are typically associated with debilitating or predisposing conditions or both. *Candida* infections are the first symptom of active AIDS to appear in HIV-positive individuals. One factor shared by AIDS patients and the other susceptible individuals described previously is hormonal imbalance resulting from HPA axis activation. Furthermore, emotionally affected women who perceive their situation to be stressful have a higher incidence of vaginal *Candida* infections (Reszel et al., 1993). Candidiasis also appears frequently in people undergoing surgery, a unique form of stress that involves emotional stressors (anxiety), chemical stressors (anesthesia), and physical stressors (surgery) (Mishra et al., 1994). Similarly, the emotional stress of divorce has been positively correlated with increased incidence of the carriage of *Candida* (Reszel et al., 1993). Such associations of stress and fungal infection in vulnerable populations are only beginning to be understood.

➤ **STRESS-IMMUNITY AND NURSING SCIENCE**

The holistic view of human nature ascribed to by the discipline of nursing is harmonious with the philosophical underpinnings of psychoneuroimmunology (McCain & Smith, 1994; Zeller, McCain, & Swanson, 1996). As a result, nurse researchers have used a psychoneuroimmunological framework in their research and have made significant contributions to the scientific growth of this field. Nurse investigators have examined stress-immune interactions in a variety of immune-based illnesses, including asthma, HIV, and cancer. In addition, nurse scientists have documented the immunosuppressive nature of postoperative pain and the effects of stress on wound healing. A psychoneuroimmunologic framework has also been used to understand the immunologic implications of child birth and postpartal stress on maternal-infant well-being. Some of these studies are addressed in the following discussion.

Asthmatic symptoms can often be initiated and potentiated by stressful life events. Kang et al. studied the effect of examination stress in asthmatic and nonasthmatic adolescents. Their results revealed that examination stress produces significant alterations in circulating immune cell subsets and in both proliferative and cytolytic activities. No differences were found between the asthmatic and nonasthmatic adolescents, however. Both healthy and asthmatic adolescents reported similar levels of stress and similar changes in immune cell numbers and function (Kang, Coe, Karaszewski, & McCarthy, 1998; Kang, Coe, & McCarthy, 1996). The lack of a relationship between asthma status and social support was believed to be due to the stability and well-managed nature of this asthmatic population (Kang, Coe, McCarthy, & Ershler, 1997). In a similar study, examination stress in adolescent asthmatics produced a bias toward a Th2-like pattern of cytokine production compared to that of nonasthmatic adolescents (Kang, Coe, McCarthy, et al., 1997). These studies are suggestive and need to be

replicated in asthmatics with less stable disease and in naturalistic situations of more intense or chronic stress or both.

The laboratory of Gayle Page conducted a series of compelling experiments in rodents that showed that untreated postoperative pain led to impaired NK cell activity and enhanced tumor metastases (Ben-Eliyahu, Page, Yirmiya, & Shakhar, 1999; Page & Ben-Eliyahu, 1997; Page, Ben-Eliyahu, & Liebeskind, 1994; Page, Ben-Eliyahu, Yirmiya, & Liebeskind, 1993). In her model, rats were subjected to laparotomy and injected with NK cell-sensitive radiolabeled tumor cells that metastasized to the lung. Rats that were treated with morphine, and that exhibited signs of pain relief, had significantly less radiolabeled tumor in the lung, fewer metastatic lesions on the lung, and higher postoperative NK cell activity (Page et al., 1993, 1994). These results suggest that untreated postoperative pain leads to impaired immune function (e.g., reduced NK cell activity) and potentially increased organ localization of tumor emboli. In addition, this research group has demonstrated that the degree of postoperative pain immunosuppression is related to the estrus stage of the rat, suggesting that reproductive hormones may affect the stress-immune response to surgical stress (Ben-Eliyahu, Page, Shakhar, & Taylor, 1996). As a whole, the studies of Page and colleagues indicate that the treatment of pain is necessary not only to alleviate suffering but also to prevent pain-induced immunosuppression and possible tumor metastatic spread. Although these observations have been made in animal models, others have shown in humans that surgery for tumor resection leads to a postoperative reduction in NK cell activity compared to preoperative levels (Pollack, Lotzova, & Stanford, 1992). Evidence that healing and recovery from surgery are potentially altered by stress has also been provided by Wysocki (1996), who found that noise stress delayed wound healing in an animal model. Also, McCarthy, Ouimet, and Daun (1991) have provided evidence that noise stress alters lymphoid cell function needed for tissue repair. The previously discussed results support the

supposition that an individual's psychological state can influence surgical recovery by altering various aspects of immunity (Kiecolt-Glaser, Page, Marucha, MacCullum, & Glaser, 1998).

Immunity and HIV Progression

Living with HIV is replete with multiple stressors, and nurse scientists have contributed to the supposition that the stress-endocrine-immune axis is implicated in HIV disease progression (McCain & Cella, 1995; McCain & Gramling, 1992; Robinson, Matthews, & Witek-Janusek, 1999a; Robinson et al., 1999b). Stress-induced neuroendocrine activation leads to elevations in plasma cortisol. *In vitro*, physiological concentrations of cortisol increase HIV replication in monocyte-derived macrophages, suggesting a potential role for stress hormones in HIV disease activation and progression (Swanson et al., 1998). The effectiveness of stress-reducing interventions in HIV disease has been evaluated by McCain et al. In a pretest-posttest design, the effect of a 6-week stress management program in HIV disease was evaluated (McCain et al., 1996). Outcome measures at 6 weeks and 6 months included perceived stress, quality of life, psychological distress, illness-related uncertainty, and CD4+ T lymphocyte levels. Although the program improved measures of emotional well-being, no significant changes in CD4+ lymphocyte levels were detected. It is likely that CD4+ lymphocyte number may not be a sensitive indicator of improvement in immune function, and other types of immunological assessment may yield more positive results.

In an ongoing intervention study, Robinson and colleagues are examining the efficacy of an 8-week, mindfulness meditation-based stress-reduction program on psychoimmune variables in HIV-positive individuals. These investigators are measuring NK cell activity, which is an important host defense mechanism against viral infections, and opportunistic microbial infections, which cause significant morbidity and mortality in HIV-positive

patients. Preliminary data from the study suggest a positive effect of this program on psychological well-being and immune status (Robinson et al., 1999a). Undoubtedly, nurse investigators will continue to explore the role of stress in HIV disease management and progression.

Stress-induced immunosuppression may have special relevance to the nursing care of cancer patients who undergo immunosuppressive therapies. The emotional stress of undergoing breast biopsy for cancer diagnosis has been clearly demonstrated and presents a useful human paradigm to study psychologic stress (Hooper, Mathews, & Witek-Janusek, 1997; Witek-Janusek & Mathews, 1999b). Anticipation of breast cancer diagnosis has been shown to alter immune cell subsets (Fillion, Lemyre, Mandeville, & Piche, 1996) and TH1 and TH2 cytokine production (Witek-Janusek & Mathews, 1999b). The effect of stress on gene transcription factors has been investigated in women undergoing diagnostic breast biopsy. Gene transcription factors play a significant role in the immune response and can regulate the production of cytokines (Wulczyn, Krappmann, & Scheidereit, 1996). In the diagnostic breast biopsy paradigm, nuclear localization in lymphocytes of two transcription factors, NF- κ B and AP-1, was decreased in women experiencing significant emotional stress, whereas when stress was relieved (post-biopsy) the nuclear localization of these gene transcription factors was similar to those of age-matched control women (Nagabhushan, Mathews, & Witek-Janusek, 2000). If and how these factors relate to the modification of immune function and to cancer outcome remain to be determined, but such studies will move this field to a molecular understanding of the effects of stress.

Nurse researchers have used a psychoneuroimmunologic approach toward understanding the impact of childbirth and postpartal stress on maternal child health. Maureen Groer and her research team at the University of Tennessee College of Nursing have demonstrated that childbirth stress leads to a reduction in maternal secretory immuno-

globulin A (sIgA). This reduction was most pronounced in women who reported an increased state of anxiety. Women with very low or undetectable levels of sIgA had a greater incidence of postpartal complications, and their infants had more illnesses. These results indicate that the stress of childbirth can have profound effects on maternal immune function, which can alter the clinical course of mothers and that of their infants (Annie & Groer, 1991). Interestingly, Groer, Mozingo, et al. (1994) demonstrated that touch, provided by a 10-minute slow-stroke effleurage back rub, was shown to increase sIgA levels in elderly adults. It remains to be determined if such an intervention may blunt the decrease in sIgA observed during parturition. Other nurse investigators have demonstrated that glucocorticoid hormones can profoundly influence the pattern of cytokine production (i.e., colony-stimulating factors) from neonatal mononuclear cells obtained from umbilical cord blood. Such immunomodulation may alter the newborn's ability to resist infectious pathogens (Witek-Janusek & Mathews, 1999b).

The unique psychologic and immunologic relationship between a breast-feeding mother and her infant is an intriguing paradigm in which to evaluate the stress-immune relationship. Stress-induced alterations in maternal immunity in breast-feeding mothers could potentially alter their capability to provide optimal levels of immunoglobulins for their infants. Postpartal mothers of preterm infants report high levels of mood disturbance compared to the general population. Mothers who score higher on negative mood subscales of the Profile of Mood States produce less milk sIgA than those who report lower negative mood states. Conversely, mothers who report higher vigor and anger produce greater amounts of milk sIgA (Groer, Droppleman, & Mozingo, 1999). Interestingly, an inverse relationship between cortisol levels and sIgA in breast milk has been reported, such that the higher milk cortisol was associated with lower milk sIgA. It is plausible that increased maternal stress leads to elevated plasma and milk cortisol. Higher levels of milk cortisol may

impair milk immune cell production of immunoglobulins or other immune cell functions or both (Groer, Humenick, & Hill, 1994; Groer et al., 1999). Such stress-induced alterations in milk endocrine and immune composition may potentially impact the immunologic benefits that infants receive from breast milk and certainly require additional investigation. This is especially relevant to premature and low-birth-weight infants, who are at high risk for infectious illness.

► FUTURE DIRECTIONS AND NURSING IMPLICATIONS

The guiding premise of psychoneuroimmunology is that stress-induced impairment of immune function influences disease progression or response to therapy or both. These types of investigations are directed toward an understanding of the effect of the psychoendocrine stress response on the immune system, particularly within the context of cancer, autoimmune disease, infectious disease, and maternal child health. Nurses must recognize the potential effectiveness of biobehavioral approaches to the care of patients with immune-based disease. Such approaches to stress management may not only improve the quality of life and emotional well-being of targeted populations but also halt disease progression or complications from opportunistic infection or both.

Complementary and alternative therapies integrate preventive and curative therapies that consider the whole person and are used to "complement" traditional approaches to illness. The use of complementary and alternative therapies by American health care consumers has markedly increased; rigorous scientific testing of such practices has lagged behind, however (Eisenberg et al., 1998; Fontanarosa & Lundberg, 1998). Increased use of massage, touch, meditation, acupuncture, yoga, botanical herbs, guided imagery, and behavioral-based stress reduction programs has spurred a renewed interest in understanding the scientific basis for such ap-

proaches toward healing and health maintenance. This integrative biobehavioral, mind-body, therapeutic approach is harmonious with the view of psychoneuroimmunology and that of nursing science.

As discussed previously, the links among one's emotional state, neuroendocrine activity, and immune response are well described. Future emphasis needs to be placed on understanding the mechanism(s) of stress-induced immune dysregulation and the relationship between stress-induced immune dysregulation and health outcome. That is, do stress-induced changes in immunity alter health, and can stress-reducing interventions that strengthen immunity halt disease progression and improve health? These are critical questions that require intensive empirical investigations using human paradigms of stress. Such approaches will lead to a better understanding of disease and to better diagnosis, treatment, or both of stress-induced immune dysfunction. The results will provide the scientific foundation that will lead to the identification of individuals "at risk" for psychological distress and altered immune reactivity. Such identification will permit the development of psychologically based interventions designed to reduce stress, promote immunocompetence, and hence improve health. Such interventions may prove to be cost-effective additions to traditional forms of treatment or therapy or both and hold promise for disease control. Ultimately, this will serve to enhance the quality and the quantity of life.

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Aberrant Nuclear Expression of AP-1 and NFkB in Lymphocytes of Women Stressed by the Experience of Breast Biopsy

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We have investigated the expression of AP-1 and NFkB in peripheral blood lymphocytes of women scheduled for breast biopsy. Samples were collected when women were informed of the need for biopsy (prebiopsy, T1, 5–7 days prior to the actual biopsy) and 7–10 days after they learned the result of their biopsy (postbiopsy, T2). At the time of blood collection, psychological stress was evaluated using Spielberger's State Trait Anxiety Inventory (STAI) and the Profile of Mood States (POMS). Women scheduled to undergo breast biopsy reported significant increases in anxiety (STAI) and mood disturbance (POMS). Gel shift mobility assays showed that mitogen stimulated peripheral blood lymphocytes of these women were less capable of the nuclear expression of AP-1 or NFkB at T1. Similar assessments, 7–10 days after the women learned of the results of their breast biopsy, showed these same women to have a marked reduction in anxiety and mood disturbance and an increased nuclear translocation of AP-1 and NFkB. These results show a significant decrease in nuclear AP-1 and NFkB expression during the period of emotional distress prior to biopsy with a return of nuclear transcription activity to normal levels when distress was relieved. Several studies have correlated increased psychological stress with decreased immune function. The results of this study suggest that psychological stress may mediate immunosuppression by altering the expression of the transcription factors, AP-1 and NFkB.

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Key Words: transcription factors; NFkB; AP-1; breast biopsy; psychological stress; human peripheral blood lymphocytes; gel shift assay; cancer.

INTRODUCTION

A number of studies have explored the relationship between psychological stress and the immune response (Glaser, Rice, Sheridan, Fertel, Stout, Speicher, Pinsky, Kotur, Post, Beck, & Kiecolt-Glaser, 1987; Whiteside & Herberman, 1994). Those studies have assessed stress and immunity in medical students during classroom examinations, caregivers of diseased patients, individuals undergoing exercise-induced stress, volunteers exposed to experimental laboratory stress, and women with breast cancer (Glaser, Rice, Sheridan, Fertel, Stout, Speicher, Pinsky, Kotur, Post, Beck, & Kiecolt-Glaser, 1987; Whiteside & Herberman, 1994; Anderson, Farrar, Golden-Kreutz, Kreutz, Kutz, Maccallum, Courtney, & Glaser, 1998). Stressed individuals showed decreased lymphoblast transformation in response to mitogens (Dobbin, Harth, McCain, Martin, & Cousin, 1991); increased Epstein-Barr virus, Herpes simplex virus, and cytomegalovirus reactivation (Bonneau, Zimmerman, Ikeda, & Jones, 1998); dysregulation of cytokines (Marshall Jr, Agarwal, Loyod, Cohen, Henninger, & Morris, 1998); elevated secretion of proinflammatory cytokines; and decreased natural killer cell activity (Mae, Song, Lina, Jongh, Van Gastel, Kenis, Bosmans, De Meester, Benoy, Neels, Demedts, Janca, Scharpe, & Smith, 1998; Whiteside & Herberman, 1994).



NFkB and AP-1 are transcription factors that regulate lymphocyte function. Nuclear expression of these transcription factors is increased during lymphocyte activation, cytokine secretion, and latent viral reactivation. Agents that are known to block lymphocyte activation are immunosuppressive and inhibit NFkB and AP-1 activity (Kopp & Ghosh, 1995). Thus we hypothesized that stress may alter the expression of these transcription factors. Experiments were designed to evaluate the expression of NFkB and AP-1 in the peripheral blood lymphocytes isolated from women pre- and postbreast biopsy. This experimental design provides a naturalistic psychological stress paradigm in which the effects of stress can be evaluated.

METHODS

Mononuclear cells were prepared from heparinized whole blood by Ficoll-Hypaque separation (Sigma, St Louis, MO). Mononuclear cells were cultured at a density of 1×10^6 /ml/well in triplicate with or without phorbol 12-myristate 13-acetate and phytohemagglutinin (PMA + PHA), for 48 h at 37°C as described previously (Witek-Janusek & Mathews, 1999). Phenotypic analysis of mononuclear cells was performed with fluoro-chrome conjugated antisera by flow cytometry with a Becton-Dickinson FACStar Plus System (Hialeah, FL). T lymphocytes were identified with anti-CD3 ϵ , B lymphocytes with anti-Ig, and NK cells with anti-CD56, from Pharmingen (San Diego, CA). The nonadherent cells (>99% lymphocytes as judged by Wright-Giemsa staining) were then harvested, treated with lysing buffer (10 mM Tris-HCl, pH 7.4, 2 mM magnesium chloride, 140 mM sodium chloride, 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, and 0.1% tritonX100), and stored at -70°C. The samples were thawed at 37°C in a water bath. The nuclear extract was prepared as described previously (Singh & Aggarwal, 1995). NFkB and AP-1 double standard oligonucleotides (Promega, Madison, WI) were end-labeled with [γ -³²P]-ATP (Amersham, Arlington Heights, IL). One microliter (30,000 cpm) of labeled probe was incubated with 3 μ l of nuclear extract (protein concentration, 5 μ g) and binding buffer (15 mM Tris-HCl, pH 7.5, 7.5% glycerol, 75 mM sodium chloride, 1.5 mM EDTA, 1.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 0.3% NP-40, and 20 μ g bovine serum albumin), final volume 25 μ l for 30 min at 25°C and separated with a 4% nondenaturing polyacrylamide gel for 1.5 h with 0.5% TBE (Singh & Aggarwal, 1995). The gels were vacuum dried for 1 h and exposed to X-ray film for 12 h at -70°C. The developed autoradiograms were densitometrically quantified with an Alphaimager 2000 version 4.03 (Innotech Corp, San Leandro, CA) video image analyzer. The specificity of shifted bands was confirmed by incubating first with a 10-fold excess of cold oligonucleotides and then with radioactively labeled oligonucleotides. Results are expressed as percentage of positive control (100%, PMA and PHA stimulated nuclear extracts from normal, nonstressed individuals) for each gel.

Sixteen women, 52.1 ± 3.7 years of age, were enrolled from the Loyola University Breast Care Center on the day that their physician recommended the need for a breast biopsy. After signing an informed consent, blood samples (16 ml) were obtained by venipuncture and the psychological assessments were administered in a private area adjacent to the clinic. At approximately 5–7 days after T1, the study women underwent breast biopsy. These same women were also sampled post biopsy (T2). T2 occurred 7–10 days after the women learned the results of their breast biopsy. At this time the women met with the study's nurse researcher at the Breast Care Center,

TABLE 1
Evaluation of Psychological Stress in Breast Biopsy Patients

Psychological test	T1, Prebiopsy	T2, Postbiopsy	Normal controls
POMS	22.0 \pm 7.1 ^a	0.2 \pm 6.9	2.2 \pm 9.3
STAI	42.7 \pm 4.4 ^a	27.8 \pm 2.2	27.2 \pm 1.7

Note. Values represent mean \pm standard error of mean. POMS, Profile Of Mood State; STAI, State Trait Anxiety Inventory; T1, Time 1, prior to breast biopsy; T2, Time 2, 7–10 days after breast biopsy results were known.

^a Statistically significant $p < .05$ from T2 and controls.

their blood was collected, and the psychological instruments were administered. Age-matched, nonstressed control women were solicited from within the University community. At a convenient time these women were met in a University office setting. In a procedure similar to that for the biopsy group, their blood was obtained by venipuncture and the same psychological instruments were administered. Control women were sampled at one time point only.

For the purposes of this study, anxiety was assessed using Spielberger's State Anxiety Inventory (STAI) and mood was assessed using the Profile of Mood States (POMS). The STAI measures the state of anxiety and it is designed to assess "state of mind" or anxiety at the moment. The STAI has alpha reliability coefficients of 0.83–0.92 and convergent validity with other anxiety instruments (alpha 0.75–0.80). The POMS is a reliable and valid tool designed to identify and assess general distress and mood. It has internal consistency reliabilities of 0.87–0.95, and stability coefficients (test–retest) of 0.65–0.74 (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1970; McNair, Lorr, & Droppleman, 1992). These instruments were used to reflect the level of psychological distress experienced by these women pre and post breast biopsy. This study was approved by the Institutional Review Board for the study of Human Subjects of Loyola University Medical Center.

RESULTS

Table 1 shows the psychological profile of women pre biopsy (T1) and post biopsy (T2). Mood disturbance and anxiety, as measured by the POMS and STAI, showed a significant ($p < .05$) decrease from pre- (T1) to postbiopsy (T2). These results demonstrated that the women experience two forms of psychological distress, anxiety and mood disturbance, at T1. At T2, reduced anxiety and improved mood were demonstrated and were not different from nonstressed, age-matched, control women. Electrophoretic mobility shift assays were used to quantify the AP-1 and NFkB activity in the stimulated peripheral blood lymphocytes of the subjects. Lymphocytes obtained at T1 exhibited lower expression of nuclear NFkB and AP-1 compared to T2 (Table 2). No change in nuclear localization of another transcription factor, Oct-1, was noted either before or after biopsy. Examples of the electrophoretic mobility shift assays are presented in Figs. 1, 2, and 3. The reduction in expression of AP-1 and NFkB was greater for NFkB than AP-1. Follow-up of these patients at the post-biopsy period (T2) showed NFkB and AP-1 activity to be higher ($p < .001$) than that observed at T1. From T1 to T2 the NFkB activity increased nearly threefold, while that of AP-1 nearly doubled. No phenotypic differences were observed in the

TABLE 2
Evaluation of Peripheral Blood Lymphocytes of Breast Biopsy Patients

Transcription factor	Percentage of Positive Control		Normal controls
	T1, Prebiopsy	T2, Postbiopsy	
NFkB	32.8 \pm 5.3 ^a	104.3 \pm 3.2	105.9 \pm 3.8
AP-1	57.4 \pm 5.1 ^a	107.8 \pm 2.7	94.7 \pm 5.1
Oct-1	101.8 \pm 4.2	102.6 \pm 3.9	100.9 \pm 5.0
Lymphocyte Population %			
T	71.8 \pm 9.0	72.2 \pm 8.1	70.7 \pm 5.6
B	16.1 \pm 5.1	14.7 \pm 4.8	17.8 \pm 5.4
NK	13.0 \pm 11.7	12.6 \pm 9.2	11.3 \pm 6.6

Note. Percentage of AP-1 and NFkB activity in the nuclear extracts of peripheral blood lymphocytes stimulated with PMA/PHA for 48 hrs. Decreased NFkB and AP-1 activity were observed prebiopsy (T1). When the stress was relieved, postbiopsy (T2), AP-1, and NFkB nuclear activity increased. A positive normal (nonstressed) control (100% activity) was examined for each set of samples for calculating relative percentage activity. Values represent mean \pm standard error of mean. No statistically significant differences were observed in the percentage of Oct-1 nuclear activity. No statistically significant differences were observed between the percentages of the individual lymphocyte populations analyzed between groups.

^a Statistically significant $p < .001$ when compared to T2 and normal controls.

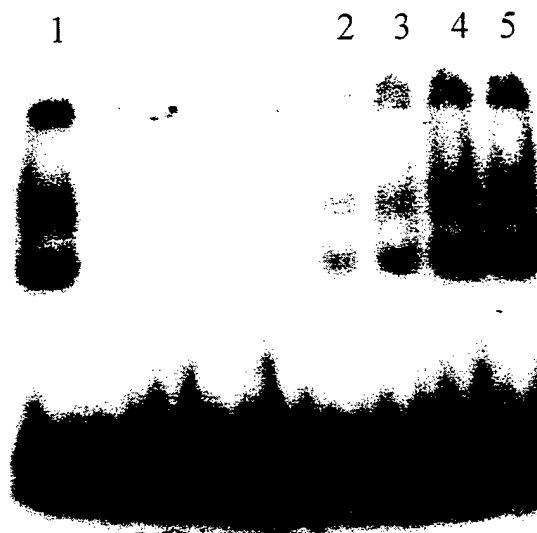


FIG. 1. Representative electrophoretic mobility shift assessment of NFkB nuclear localization in extracts from the lymphocytes of a woman pre- and postbiopsy. Lane 1 contains the PMA and PHA stimulated nuclear extract from the lymphocytes of a normal, nonstressed individual. Lanes 2 and 3 contain the PMA and PHA stimulated nuclear extracts from lymphocytes of a woman prior to biopsy. Lanes 4 and 5 contain the PMA and PHA stimulated nuclear extracts from lymphocytes of the same woman postbiopsy. Lanes 2–5 are extracts derived from the lymphocytes of one woman. Densitometric quantification of the autoradiographic results were calculated for both of the bands in each lane. The results for the higher molecular weight band are presented in Table 2.

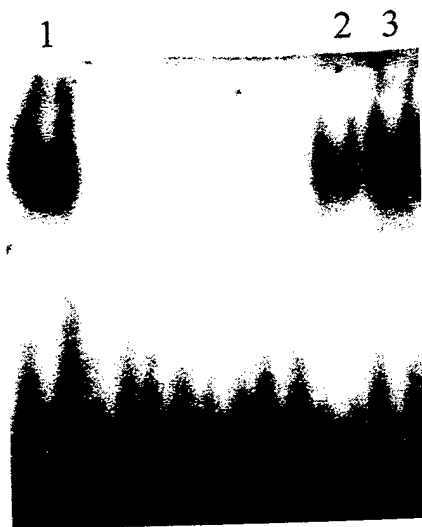


FIG. 2. Representative electrophoretic mobility shift assessment of AP-1 nuclear localization in extracts from the lymphocytes of a woman pre- and postbiopsy. Lane 1 contains the PMA and PHA stimulated nuclear extract from the lymphocytes of a normal, nonstressed individual. Lane 2 contains the PMA and PHA stimulated nuclear extract from lymphocytes of a woman prior to biopsy. Lane 3 contains the PMA and PHA stimulated nuclear extract from lymphocytes of the same woman postbiopsy.

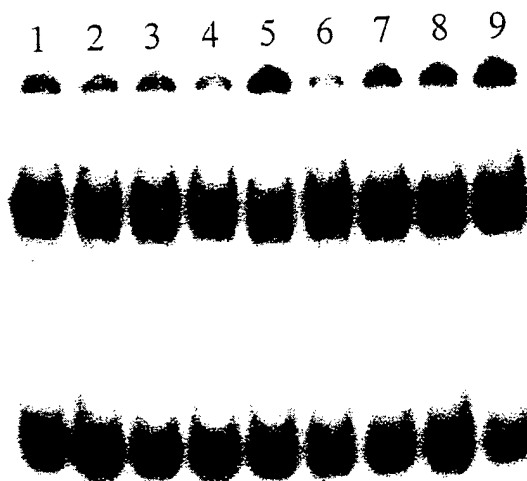


FIG. 3. Representative electrophoretic mobility shift assessment of Oct-1 nuclear localization in extracts from the lymphocytes of women pre- and postbiopsy. Lane 1 contains the PMA and PHA stimulated nuclear extract from the lymphocytes of a normal, nonstressed individual. Lanes 2, 3, 4, and 5 contain the PMA and PHA stimulated nuclear extracts from lymphocytes of women prior to biopsy. Lanes 6, 7, 8, and 9 contain the PMA and PHA stimulated nuclear extracts from lymphocytes of women postbiopsy. Lanes 2-9 are extracts derived from the lymphocytes of four women and are presented sequentially with the following pairings (Lane 2) pre- and (Lane 6) postbiopsy from the same woman, (Lane 3) pre- and (Lane 7) postbiopsy from the same woman, etc.

women's lymphocyte populations from T1 to T2. The activity of the transcription factors in women after the experience of breast biopsy was similar to that of age-matched and nonstressed women.

DISCUSSION

We have examined nuclear localization of two transcription factors, NFkB and AP-1, in lymphocytes isolated from the peripheral blood of women scheduled to undergo biopsy for suspected breast cancer. The blood samples were collected at two different time intervals, before and after breast biopsy. The prebiopsy sampling occurred on the day the woman's physician informed her of the need for a breast biopsy. At this time the women exhibited increased anxiety and mood disturbance, which was most likely related to the uncertainty associated with an impending biopsy of the breast. From pre to postbiopsy there was a clear decrease in the anxiety and mood disturbance expressed by these women. These changes in anxiety and mood disturbance were reflected by changes in NFkB and AP-1. The nuclear levels of these transcription factors were decreased prebiopsy or during the time of heightened psychological stress; while at postbiopsy both the psychological stress and the transcription factors returned to that of nonbiopsied control women.

Experimental studies in animals and clinical studies in humans suggest that psychological stress decreases immune function and increases the risk for infectious disease and the reactivation of latent viruses. NFkB regulates the expression of gene products such as cytokines, acute phase proteins, and adhesion molecules. These are necessary components of immunity which ensure effective protection from infectious disease (Dobbin, Harth, McCain, Martin, & Cousin, 1991; Bonneau, Zimmerman, Ikeda, & Jones, 1998; Mae, Song, Lina, Jongh, Van Gastel, Kenis, Bosmans, De Meester, Benoy, Neels, Demedts, Janca, Scharpe, & Smith, 1998; Kopp & Ghosh, 1995). It is possible that a stress-induced decrease in NFkB and AP-1 activity may down regulate the secretion of important cytokines that promote an effective immune response and are required for optimal protection from infection. This is especially important for cancer patients undergoing immunosuppressive chemotherapy. Decreased immunity in cancer patients may not only increase susceptibility to infection but may also accelerate the growth and progression of cancer (Cohen & Rabin, 1998). In a recent study, reduced translocation of lymphocyte transcription factors was observed in women with breast cancer. Those authors speculated that a defect in transcription factor translocation may be related to breast cancer (Kurt, Urba, Smith, & Schoof, 1998). Although the design of this study does not permit a causal interpretation, the data does suggest that the emotional distress associated with impending breast biopsy may influence the capacity of lymphocytes to translocate nuclear transcription factors. Therefore, psychological stress may be an important modulator of transcription factors which, in turn, may result in reduced immune function at the level of transcription.

NFkB, AP-1, and other transcription factors act together to regulate and activate components of the immune system. To our knowledge, this is the first report suggesting a possible molecular mechanism whereby psychological stress may modulate immune function. Because NFkB, AP-1, and related transcription factors play a central role in the immune system (Kopp & Ghosh, 1995; Thomas, Tymms, McKinlay, Shannon, Seth, & Kola, 1997), they may be a marker or means by which to measure the overall effect of stress upon immune function.

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Immunological and Psychological Analysis of Women Experiencing Breast Biopsy

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ABSTRACT

Biopsy of the breast for cancer diagnosis is an emotional experience, characterized by anxiety and fear. This experience may impair immune responses. This study evaluated a woman's immunological and psychological response pre and post breast biopsy. Perceived stress, anxiety, and mood disturbance were heightened pre-biopsy. Post-biopsy; perceived stress, anxiety, and mood disturbance decreased but did not return to levels reported by non-biopsied control women. Natural killer cell activity was significantly depressed pre and post biopsy, when compared to non-biopsied, control women. Post biopsy, natural killer cell activity was less than that exhibited pre biopsy. No changes in number of NK cells were observed at either pre or post biopsy time points. Production of $\text{INF}\gamma$ was significantly reduced pre and post biopsy and production of IL-4, IL-6, and IL-10 were significantly increased by the experience of breast biopsy. Thus, impending breast biopsy is marked by increased perceived stress, anxiety, and mood disturbance, which is relieved post-biopsy, but does not return to levels reported by non-biopsied women. Associated with the stress of breast biopsy was depressed natural killer cell activity and altered cytokine production.

Key words: Psychological stress, mood disturbance, breast biopsy; breast cancer; human peripheral blood lymphocytes; natural killer cells; IL-2; IL-4; IL-6; IL-10; interferon γ

INTRODUCTION

The experience of breast cancer diagnosis is a time of considerable uncertainty, anxiety, and emotional distress (Northouse, L. L., Jeffs, M., Cracchiolo-Caraway, A., Lampman, L., & Dorris, G., 1995; Deane, K. A. & Degner, L. F., 1998). This emotional experience often begins with the discovery of clinical findings that indicate the need for biopsy of the breast (Benedict, S., Williams, R. D., & Baron, P. L., 1994). In the United States, approximately 1.5 million women undergo breast biopsy annually (Venta, L. A., 2000). Biopsy of the breast and the concomitant uncertainty and fear of a cancer diagnosis is stressful (Nagabhushan, M., Mathews, H. L., & Witek-Janusek, L., 2001; O'Mahony, 2001). Women experience high levels of anxiety after the discovery of a breast lump (MacFarlane, M. E. & Sony, S. D., 1992) and at the time of biopsy they report higher levels of stress compared to patients awaiting general surgery (Hughson, A. V., Cooper, A. F., McArdle, C. S., & Smith, D. C., 1988). Reciprocal neuro-chemical pathways and shared receptor systems connect the nervous, endocrine, and immune systems (Madden, K. S. & Felten, D. L., 1995; Weigent, D. & Blalock, J., 1999) and this multi-system network is thought to maintain homeostasis in the presence of changing demands from the external environment. This intricate communication network provides the link whereby perceived environmental stressors or demands, such as biopsy of the breast, may effect the immune system and influence the course of disease (Witek-Janusek, L. & Mathews, H., 2000).

A large body of evidence has documented the impact of psychosocial stress on the human immune response (Hillhouse, J. E., Kiecolt-Glaser, J. K., & Glaser, R., 1991). Stress-induced immunosuppression accompanies a variety of acute and chronic life stressors such as bereavement (Irwin, M., Daniels, M., Bloom, E. T., Smith, T. L., & Weiner, H., 1987), depression (Weisse, C. S., 1992), marital conflict (Kiecolt-Glaser, J. K., Malarkey, W. B., Chee, M., Newton, T., Cacioppo, J. T., Mao, H. Y., & Glaser,

R., 1993), academic exam stress (Kiecolt-Glaser, J. K., Glaser, R., Strain, E. C., Stout, J. C., Tarr, K. L., Holliday, J. E., & Speicher, C. E., 1986), and caregiving in chronic disease (Kiecolt-Glaser, J. K., Glaser, R., Shuttleworth, E. C., Dyer, C. S., Ogrocki, P., & Speicher, C. E., 1987). Several studies have examined the relationship between stress and natural killer cell activity (NKCA) and these studies have shown that stress can influence NKCA (Trinchieri, G., 1989). Andersen *et al.* (Andersen, B. L., Farrar, W. B., Golden-Kreutz, D., Kutz, L. A., MacCallum, R., Courtney, M. E., & Glaser, R., 1998) studied the stress-immune response of 116 women who were diagnosed with invasive breast cancer (Stage II and III). Women were enrolled within four months of their breast surgery but prior to adjuvant therapy initiation. The results of that study indicated that higher stress levels were predictive of lower NKCA, diminished natural killer (NK) cell response to IFN γ , and decreased lymphocyte proliferation (Andersen, B. L., Farrar, W. B., Golden-Kreutz, D., Kutz, L. A., MacCallum, R., Courtney, M. E., & Glaser, R., 1998). It is possible that stress may influence cancer control. Although a direct relationship between NKCA and cancer has not been equivocally established, patients with a variety of solid tumors (e.g., breast, cervix, endometrium, ovary, lung) do exhibit reduced NKCA (Pross, H. F. & Lotzova, E., 1993). Recent data, albeit in gene-depleted mice, provides evidence that NK cells mediate protection from tumor initiation (van den Broek, M. E., Kagi, D., Ossendorp, F., Toes, R., Vamvakas, S., Lutz, W. K., Melief, C. J., Zinkernagel, R. M., & Hengartner, H., 1996; Street, S. E., Cretney, E., & Smyth, M. J., 2001), primary tumor growth (van den Broek, M. E., Kagi, D., Ossendorp, F., Toes, R., Vamvakas, S., Lutz, W. K., Melief, C. J., Zinkernagel, R. M., & Hengartner, H., 1996), and tumor metastasis (Smyth, M. J., Thia, K. Y., Cretney, E., Kelly, J. M., Snook, M. B., Forbes, C. A., & Scalzo, A. A., 1999; Street, S. E., Cretney, E., & Smyth, M. J., 2001).

The effects of stress upon the immune system extends not just to NK cells but also to the production of cytokines. Heightened levels of stress are related to decreased synthesis of IFN γ (Glaser, R., Rice, J.,

Speicher, C. E., Stout, J. C., & Kiecolt-Glaser, J. K., 1986) and a poorer NK response to IFN γ and IL-2 has been observed in stressed individuals compared to non-stressed individuals (Fawzy, F. I., Kemeny, M. E., Fawzy, N. W., Elashoff, R., Morton, D., Cousins, N., & Fahey, J. L., 1990). This effect was independent of both the percentage of NK and NK surface receptor density. Others have reported that posttraumatic stress disorder (Maes, M., Smith, R., & Scharpe, S., 1995) and academic exam stress (Marshall, G. D., Jr., Agarwal, S. K., Lloyd, C., Cohen, L., Henninger, E. M., & Morris, G. J., 1998) also lead to cytokine dysregulation. Moreover, stress reduction interventions modulate NKCA and cytokine responsiveness (Esterling, B. A., Kiecolt-Glaser, J. K., Bodnar, J. C., & Glaser, R., 1994), suggesting an interactive relationship between stress and NK function that may be mediated in part by cytokines (Esterling, B. A., Kiecolt-Glaser, J. K., & Glaser, R., 1996). Cytokine dysregulation in response to stress is believed to be triggered by an increase in adrenal cortisol secretion and a decrease in adrenal DHEA and DHEAS secretion (Schwartz, M. D., Lerman, C., Miller, S. M., Daly, M., & Masny, A., 1995). During stress, adrenal steroid hormone metabolism shifts such that DHEA/DHEAS production decreases and cortisol increases (Regelson, W., Loria, R., & Kalimi, M., 1994). This shift in adrenal steroid hormone profile is recognized as having an important immunomodulatory effect, which by altering Th1/Th2 cytokine balance, leads to decreased production of IFN γ (a Th1 cytokine) and enhanced production of IL-4 (a Th2 cytokine) (Agarwal, S. K. & Marshall Jr., G. D., 2001). Such a change in cytokine balance, characterized by low levels of IFN γ , can depress NK cell function (Targan, S. & Dorey, F., 1980). The purpose of this study was to investigate the experience of breast biopsy for breast cancer diagnosis, as a human paradigm in which coherent links between a woman's psychological and immunological states could be evaluated. Psychological stress and components of the immune response were measured before and after breast biopsy and compared to a group of control women not undergoing breast biopsy.

METHODS

Subjects and Procedure

Women (N=48) were enrolled from the Breast Care Center of a large University based Cancer Center.

Women were studied at pre-biopsy and at post-biopsy. At pre-biopsy women were evaluated at the time of initial consultation at the Breast Care Center, when they learned that a breast biopsy was needed. This was typically 5-10 days prior to the actual biopsy procedure. Post biopsy data was collected 7-14 days after the woman learned the results of her biopsy. At each time period the participants had their blood drawn (24 ml) by venipuncture and completed the psychological measures as well as a health history questionnaire. Data collection took approximately 30 minutes.

Women who were recommended for breast biopsy (excluding fine needle aspirates) were eligible subjects. Only women with benign biopsy results are included in this report. Exclusionary criteria included: pregnancy, recent history of major psychiatric disorder, concurrent major immune-based disease, and active substance abuse. Women within 5 years of a cancer diagnosis were also excluded. Women in the biopsy group had a mean age of $53.1 \pm$ years, with a range of 18-85 years of age. A group of control women, not undergoing breast biopsy were enrolled from the University campus. Similar exclusionary criteria were applied to these women. A total of 47 women served as control subjects, with a mean age of $42.6 \pm$ years (range = 24-61 years). Data was collected from the control women at one time period only. This study was approved by the Loyola University Medical Center Institutional Review Board for the Study of Human Subjects and informed consent was obtained at the initial data collection period.

Psychological Measures

For the purposes of this study the psychological stress of breast biopsy was conceptualized as an external

event that is appraised and perceived as a threat to the individual's well-being and leads to feelings of anxiety and mood disturbance. With this in mind, the psychological assessment of women experiencing breast biopsy included measurement of perceived stress (Perceived Stress Scale and a Visual Analogue Scale), anxiety (State-Trait Anxiety Inventory), and mood state (Profile of Mood States).

Perceived Stress Scale (PSS). The PSS is a 10-item scale that is widely used as a general appraisal index of stress (Monroe, S. & Kelley, J., 1995). It measures the degree to which experiences are appraised as uncontrollable. Internal consistency is good with coefficient alphas ranging from 0.75-0.86. Test-retest reliability has been reported to be 0.85 (Cohen, S. & Williamson, G. M., 1988).

Profile of Mood States (POMS). The POMS is a 65-item measure designed to identify and assess general distress/mood. A total mood score and six sub-scores for mood were obtained. The subscores were tension, depression, anger, vigor, fatigue, and confusion. Internal consistency alphas range from 0.87 - 0.95, and stability coefficients (test-retest) are 0.65 - 0.74 (McNair, D. M., Lorr, M., & Droppleman, L. F., 1992).

Spielberger State-Trait Anxiety Inventory (STAI). The STAI is a 40-item self-report measure of state and trait anxiety. Only the state anxiety inventory was used in this study. The STAI has alpha reliability coefficients of 0.83-0.92 and convergent validity with other anxiety tools ($\alpha = 0.75-0.80$) (Spielberger, C. D., Gorsuch, R. C., & Lushene, R. E., 1970). The STAI has been used to discriminate anxiety in women with benign versus malignant breast biopsy findings (Sachs, G., Rasoul-Rockenschaub, S., Aschauer, H., Spiess, K., Gober, I., Staffen, A., & Zielinski, C., 1995).

Stress Visual Analogue Scale (VAS). The Stress VAS consists of a straight horizontal line 10.0 cm in

length, anchored on each end by labels indicating extreme limits of stress. The subject indicated the intensity of their stress by marking the appropriate place on the line that corresponded to their perception of stress. It has been positively correlated with both the STAI and the PSS, providing evidence of criterion validity (Cella, D. F. & Perry, S. W., 1986).

Immune Measures

Isolation of Peripheral Blood Mononuclear Cells. Blood was collected in sterile heparinized tubes and processed immediately. Heparinized peripheral blood was overlaid onto Ficoll/Hypaque and centrifuged at 1000 x g for 20 min. The peripheral blood mononuclear cells (PBMC) at the interface were washed twice with Hank's Balanced Salt Solution (HBSS) prior to assessment of NKCA, NK phenotype, or cytokine production. Phenotypic analysis was as described previously (Nagabhushan, M., Mathews, H. L., & Witek-Janusek, L., 2001).

Natural Killer Cell Activity. K562 tumor cells maintained *in vitro* were washed once in RPMI 1640 (GIBCO, Laboratories Grand Island, NY), pelleted by centrifugation at 500 x g for 10 min, and resuspended in approximately 0.1 ml of culture medium. 100 μ Ci of [51 Cr] (New England Nuclear, Boston, MA) was added to 1×10^7 cells in a final volume of 0.2 ml. The cells were incubated at 37°C with 5% CO₂ for 1 hr with agitation every 10 min. These procedures are as described previously (Beno, D. W., Stover, A. G., & Mathews, H. L., 1995). The cells were washed 4 times in HBSS, resuspended to 5×10^5 cells/ml in RPMI 1640 (GIBCO, Laboratories Grand Island, NY), and 0.01 ml (5×10^3) aliquoted to each well of a 96 well, round bottom assay plate (Corning Glass Works, Corning, NY). Radiolabeled cells were incubated for 4 hr with PBMC. Following incubation the supernatants were removed using a Skatron harvesting press (Skatron Inc., Sterling, VA) and the associated radioactivity was determined. Maximum release was obtained by adding 0.05% Nonident P40 (Sigma Chemical Co., St. Louis, MO).

Results are expressed as % cytotoxicity and calculated by the formula:

$$\% \text{ Cytotoxicity} = \frac{(\text{experimental DPM}^*) - (\text{minimum DPM})}{(\text{maximum DPM}) - (\text{minimum DPM})} \times 100.$$

All experimental means were calculated from triplicate values. Lytic units (LU) were calculated by a program written by David Coggins, FCRC, Frederick, MD and represents the number of cells per 10^7 effectors required to achieve 20% lysis of the targets. *DPM=disintegrations per minute.

Tumor Cell Line. The K562 tumor cells were obtained from the American Type Culture Collection, Rockville, MD. K562 cells were maintained in suspension cultures *in vitro* in Corning 25 cm² tissue culture flasks (Corning Glass Works, Corning, NY) in RPMI 1640 (GIBCO, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS) low LPS; (GIBCO, Grand Island, NY), 100 units/ml penicillin, 100 ug/ml streptomycin (Whittaker M. A. Bioproducts, Walkersville, MD), 0.1 mM non-essential amino acids, and 2 mM L glutamine (GIBCO, Grand Island, NY).

Evaluation of PBMC for Cytokine Production. Cytokines were measured under optimal conditions in bulk PBMC culture supernatant fluids as described previously (Witek-Janusek, L. & Mathews, H., 1999). PBMC (1×10^6 cells/ml) were cultured with and without PMA/PHA (PMA @ 20 ng/well; PHA @ 0.05%/well) in 24 well plates for 48 hr at 37⁰ C. Aliquots of the culture supernatants were stored at -70⁰ C for subsequent cytokine analysis.

Cytokine Measurement (ELISA). All cytokines were measured using quantitative sandwich enzyme

immunoassay techniques (Quantikine kits, R & D Systems). Sensitivities for cytokines were; IL-2 < 7 pg/ml, IL-6 < 0.7 pg/ml, IFN γ < 3 pg/ml, IL-10 < 2 pg/ml, and IL-4 < 4.1 pg/ml).

Statistical Analysis –All data is expressed as means with the standard error of the mean (SEM).

Differences between biopsy and control groups were determined by independent student t-tests; paired t-tests were used to determine differences from pre to post biopsy. A two-sided alpha of 0.05 was set for statistical significance. The Statistical Package for Social Sciences (SPSS: version 9.0) was used for data analysis.

RESULTS

Psychological Assessments

In this study both the psychological and immunological response to the experience of breast biopsy have been evaluated. To avoid the potential confounding effects of malignancy upon the immunological data, the results are presented only for women who were found to have no malignancy. In Figure 1. levels of psychological stress, as measured by the VAS and the PSS, are illustrated for the pre and post biopsy groups, as well as for that of non-biopsied, control women. The results indicate that pre biopsy was a time of heightened stress. Both the VAS and the PSS were elevated in the biopsy group relative to control women. In the case of the VAS, women reported stress to be relieved post biopsy with the results not differing from control women. Although there was a decrease in the post-biopsy PSS levels, it was not significant. PSS levels, at post-biopsy, remained similar to that reported pre biopsy and significantly greater than that reported by women in the control group. Similar results to those of the PSS were obtained by assessing mood state using the POMS and state anxiety using the STAI. The POMS total mood disturbance score (TMD) and the STAI were significantly elevated pre biopsy compared to control, non-biopsied, women, Figure 2. Although a reduction in both TMD and STAI occurred from pre to post biopsy, post-biopsy levels of both TMD and STAI remained significantly greater than that observed in the control women. As expected, no pre to post biopsy differences were observed in trait anxiety as measured by the STAI. Data not shown. These results show that the PSS, POMS, and STAI tools detected significant stress associated with the experience of biopsy that lasted beyond the actual biopsy procedure and beyond the time that the women learned of their biopsy results.

The POMS is comprised of 6 subscales and these results are presented in Figure 3. Each of these

subscales showed that pre biopsy, women experienced significantly more tension, depression, anger, fatigue, and confusion than did control women. For each of these subscales, post biopsy values were not significantly different from control women. At both the pre and post biopsy time periods women showed significantly reduced vigor compared to control women. Levels of vigor did not differ in women between pre and post biopsy time periods.

Immunological Assessments

PBMC were evaluated for the production of Th1, Th2, and proinflammatory cytokines, as well as for NKCA. In Figure 4, the comparative production of IL-2, IL-6, and IFN γ is presented. The production of IL-2 was unchanged from pre to post biopsy and was similar to that of control subjects. IFN γ production was numerically reduced pre and post biopsy, when compared to production by control subjects. The reduction was significant at both the pre and post biopsy time periods. In contrast, the production of IL-6 was significantly greater from PBMC of women at both the pre and post biopsy time periods when compared to that of control women. Although IL-6 decreased from pre to post biopsy, this reduction was not significant. Much like IL-6, the amount of produced IL-4 and IL-10 was significantly increased prior to biopsy, when compared to control values. See Table 1. Post biopsy, IL-4 levels were not significantly different from control values, while IL-10 production remained significantly elevated compared to control production of the cytokine. No significant difference was observed pre to post biopsy for IL-10 production. No cytokines were detected in the serum of representative control or biopsy subjects. Data not shown.

NKCA for the PBMC of women pre and post breast biopsy is illustrated in Figure 5. In comparison to non-biopsied control women, NKCA was significantly reduced for both the pre and post biopsy time

periods when compared to NKCA of women in the control group. Phenotypic analysis for circulating NK cells showed no differences between normal control subjects and either pre or post biopsy patients. See Table 2.

DISCUSSION

Suspicious breast findings that indicate the need for a breast biopsy herald the onset of an emotional experience that is often accompanied by uncertainty and fear of a cancer diagnosis (Benedict, S., Williams, R. D., & Baron, P. L., 1994; Northouse, L. L., Jeffs, M., Cracchiolo-Caraway, A., Lampman, L., & Dorris, G., 1995; Deane, K. A. & Degner, L. F., 1998). For most women, the unfolding of this emotional scenario occurs over a period of weeks, from the time of discovery of questionable breast findings, through the scheduling and conduct of the biopsy, and finally till the time that biopsy results are learned. This emotional experience presents a unique opportunity to understand the psycho-immune dynamics of a potentially threatening event. In this study women were evaluated at the time that they were informed a biopsy was necessary (pre biopsy) and again 1-2 weeks after they had learned the result of their biopsy (post biopsy). The results definitively show that the anticipation of breast biopsy and the possibility of a cancer diagnosis lead to a psychological state marked by heightened perceived stress, elevated state anxiety, and considerably more mood disturbance than that of control women not experiencing breast biopsy. Others have reported that breast biopsy is accompanied by emotional distress, uncertainty and anxiety. However, most of these studies either used a retrospective approach and/or did not assess the pre-post dynamics of the breast biopsy experience (Hughson, A. V., Cooper, A. F., McArdle, C. S., & Smith, D. C., 1988; Benedict, S., Williams, R. D., & Baron, P. L., 1994; Northouse, L. L., Jeffs, M., Cracchiolo-Caraway, A., Lampman, L., & Dorris, G., 1995; Deane, K. A. & Degner, L. F., 1998). The design of this study and the psychoneuroimmunologic approach provide valuable insights about women and their response to a threatening event.

The marked elevations in the levels of state anxiety reported by women at pre biopsy underscore the

emotional intensity that an impending breast biopsy evokes. Although state anxiety decreased from pre to post biopsy, the heightened level of anxiety extended post biopsy and remained significantly higher than that reported by control women. Similarly, perceived stress as measured by the PSS, was not only significantly increased at the pre biopsy time period, but also remained elevated at post biopsy compared to the control women. Moreover, the results of this study clearly show that the stress of an approaching breast biopsy is also accompanied by significant mood disturbance. A pattern similar to that observed for anxiety and perceived stress was seen for total mood disturbance. However, even though total mood disturbance significantly decreased from pre to post biopsy, women continued to report higher levels of mood disturbance at post biopsy compared to control women. This occurred despite the knowledge that their breast biopsy revealed benign findings. An examination of the subscales of mood measured by the POMS revealed that all negative mood states contributed to the total mood disturbance reported by women pre biopsy. Interestingly, vigor was significantly less at pre biopsy than that reported by control women and remained lower at post biopsy than that of control women. Vigor is a multi-dimensional concept with physical and psychological dimensions, and as such, women may report lower levels of vigor consequent to the presence of anxiety, tension, and depression. The persistence of elevated anxiety, heightened perceived stress, and increased mood disturbance post biopsy was unexpected, especially since these women were aware that the result of their biopsy was benign. Although the design of this study does not permit an understanding of the impetus for the persistence of this stress response, the results suggest that these women have not completely resolved the psychological impact of the biopsy experience.

To our knowledge, no other study has used a psychoneuroimmunologic framework to evaluate the effect of the stress of breast biopsy on NKCA and cytokine production. Th1, Th2, and proinflammatory cytokine production fell into three distinct categories for the women in the breast biopsy group. Production either remained unchanged (IL-2), showed an increase compared to control subjects (IL-4, IL-

6, and IL-10), or alternatively showed a reduction when compared to control subjects (IFN γ). IL-2 has been characterized as a Th1 cytokine and falls into the first category. Its' production by PBMC was unchanged from pre to post biopsy and was approximately the same as that of control subjects. IL-6 is a pro-inflammatory cytokine that falls into the second category. Its' production by immune cells from biopsied women was greater than control subjects with increased levels of production, pre and post biopsy. Similarly, production of the Th2 cytokines, IL-4 and IL-10, was increased in the biopsy group compared to control women. Production of IFN γ , a Th1 cytokine, was less than that of the control group. In a similar manner NKCA was also reduced in the biopsy group compared to the control group of women. Women entered into the study were all aware that breast biopsy was impending and as described above, experienced psychological stress and mood disturbance pre biopsy. The impact of psychological stress upon NKCA appeared early (pre biopsy) and, similar to the psychological stress, continued into the post biopsy period. The pre and post biopsy time point showed a significant diminution in mean NKCA compared to control women. Because the reduction in NKCA was not accompanied by a change in the number of circulating NK cells, these data clearly suggest that the observed reduction in NKCA is a consequence of reduced NKCA and not a reduction in NK cell number. It is noteworthy, that despite knowing that their biopsy findings were benign, mood, anxiety, and perceived stress of the biopsied women did not return to the levels reported by non-biopsied women. Therefore, it is likely that these women, as a group, continued to experience psychological distress, which had effects upon the immune system as judged by reduced NKCA and IFN γ production.

IL-2 is known to increase NKCA and NK cell proliferation (London, L., Perussia, B., & Trinchieri, G., 1986) and IFN γ is known to enhance NK cell cytotoxicity (Vose, B. M., Riccardi, C., Bonnard, G. D., & Herberman, R. B., 1983). It is clear from the data that NKCA is decreased in the women undergoing breast biopsy, while IL-2 production remained the same as that observed in control women, suggesting that the

subjects had the capacity to produce IL-2 but this capacity did not impact NKCA. However, IL-2 also stimulates lymphocytes to secrete cytokines, including IFN γ (Oppenheim, J. J., Ruscetti, F. W., & Faltynek, C., 1994). Increased levels of IFN γ are known to increase NKCA, resulting in more efficient lysis of target cells and enhanced recruitment of pre-NK (Targan, S. & Dorey, F., 1980). The production of IFN γ is decreased in these women and may relate to the observed reductions in NKCA. Stress can down-regulate NKCA and modulate IFN γ and IL-2 synthesis (Levy, S., Herberman, R., Lippman, M., & d'Angelo, T., 1987). Heightened levels of stress are related to decreased synthesis of IFN γ by lymphocytes from healthy subjects (Glaser, R., Rice, J., Speicher, C. E., Stout, J. C., & Kiecolt-Glaser, J. K., 1986). A poorer NK response to IFN γ and IL-2 was observed in stressed individuals compared to non-stressed individuals (Fawzy, F. I., Kemeny, M. E., Fawzy, N. W., Elashoff, R., Morton, D., Cousins, N., & Fahey, J. L., 1990). Stress reduction interventions modulate NKCA and cytokine synthesis (Esterling, B. A., Kiecolt-Glaser, J. K., Bodnar, J. C., & Glaser, R., 1994) suggesting that the link between stress and NK function that may be mediated, in part, by cytokines (Esterling, B. A., Kiecolt-Glaser, J. K., & Glaser, R., 1996). Persons experiencing chronic stress have a more activated HPA axis compared to non-stressed individuals, demonstrable as chronically elevated levels of cortisol and catecholamines (McCarty, R., Horwatt, K., & Konarska, M., 1988). This chronic activation may lead to greater concentrations of HPA products in lymphoid sites where NK cells reside and functional NKCA may be reduced. Alternatively, chronically activated NK cells may be less capable of stimulation to an effective anti-tumor state. Hence, stress activation of the HPA may down regulate NKCA by altering cytokine balance (e.g., decreased IFN γ) and or responsiveness of NK cells to cytokines. It is noteworthy, that stressed-induced changes in cortisol and in DHEA can shift the T-helper balance to a Th2 response, which would not support NKCA (Daynes, R. A., Dudley, D. J., & Araneo, B. A., 1990; Regelson, W., Loria, R., & Kalimi, M., 1994). This shift in adrenal steroid hormone profile can have an important effect by altering Th1/Th2 cytokine balance. Hence, it has been hypothesized that stress-induced changes in adrenal hormone secretion drive a switch from a Th1 to a

Th2 response. Such a change in cytokine balance, characterized by low levels of IFN γ and IL-2, can depress NK cell function (Targan, S. & Dorey, F., 1980). The data described herein does not fully support this concept in that one Th1 cytokine, IFN γ , was reduced concomitant with reductions in NKCA. The findings presented are surprising in that the production of IL-2 does not change, while the production of IFN γ is diminished throughout the entire time period of assessment for the biopsied women.

An alternative explanation for the cytokine data is possible. Lymphocytes and the cytokines they produce can be placed into naïve, central memory, or effector memory subsets (Sallusto, F., Lenig, D., Forster, R., Lipp, M., & Lanzavecchia, A., 1999). Naïve and central memory lymphocytes primarily produce IL-2 upon short-term stimulation (e.g. the 48 hr period employed in this study). In contrast, and under similar conditions, effector memory lymphocytes produce IFN γ , IL-4, and IL-10. It is possible that the effect of the stress of breast biopsy is primarily upon lymphocytes that are capable of immediate production of these effector cytokines. Specifically, the capacity of effector memory lymphocytes to elaborate their cytokines may be markedly affected by the experience of breast biopsy. No effect was observed upon IL-2 production, which is produced either by naïve or central memory lymphocytes. This interpretation is consistent with the data presented herein and it could be that a 48-hr period of culture provides for an analysis of only those lymphocytes capable of producing cytokines quickly. Finally, it is also possible that in an antigen non-specific stimulation system (PMA/PHA employed in this study), available IL-2 is consumed by the activated lymphocyte populations. This alternative explanation cannot be ruled out at this time and investigations are underway to consider this possibility.

With regard to IL-6, this cytokine is produced by a variety of cell populations and is associated with inflammatory events. The data described herein show that IL-6 is markedly increased in its production during the assessed time periods. The significance of this observation remains unknown but is consistent

with the previously demonstrated elevation in this cytokine during periods of stress (Maes, M., Smith, R., & Scharpe, S., 1995; Watkins, L. R., Nguyen, K. T., Lee, J. E., & Maier, S. F., 1999). Much like IL-6, IL-4 and IL-10 were significantly increased in the biopsied women compared to the non-biopsied control women. This increase in IL-4 and IL-10 is similar to other reports that have demonstrated stress-associated shifts in Th1/Th2 cytokine balance toward a Th2 type of response (Marshall, G. D., Jr., Agarwal, S. K., Lloyd, C., Cohen, L., Henninger, E. M., & Morris, G. J., 1998). The most dominant factor in the regulations of the Th1/Th2 cytokine balance is the cytokine milieu of the immune response. IFN γ promotes the development of Th1 responses and inhibit Th2 responses (Maggi, E., Parronchi, P., Manetti, R., Simonelli, C., Piccinni, M. P., Rugiu, F. S., De Carli, M., Ricci, M., & Romagnani, S., 1992; Manetti, R., Parronchi, P., Giudizi, M. G., Piccinni, M. P., Maggi, E., Trinchieri, G., & Romagnani, S., 1993). In contrast, IL-4 promotes the development of a Th2 response and IL-4 and IL-10 inhibit the development of a Th1 response (Parronchi, P., De Carli, M., Manetti, R., Simonelli, C., Sampognaro, S., Piccinni, M. P., Macchia, D., Maggi, E., Del Prete, G., & Romagnani, S., 1992; Manetti, R., Parronchi, P., Giudizi, M. G., Piccinni, M. P., Maggi, E., Trinchieri, G., & Romagnani, S., 1993; Rennick, D., Hunte, B., Holland, G., & Thompson-Snipes, L., 1995). Our data are consistent with the concept that a Th2 response is a default pathway, occurring in the absence of a Th1 response, when the production of IFN γ is reduced (Agarwal, S. K. & Marshall Jr., G. D., 2001). These conditions could permit the increased production of Th2 cytokines resulting in further inhibition of Th1 responses and possibly NKCA.

These results provide evidence that the psychological stress of breast biopsy for cancer diagnosis, leads to prolonged periods of perceived stress, anxiety, and mood disturbance that are conceivably associated with depressed NKCA and an altered pattern of cytokine production. It appears that stress-induced alterations in the immune system are not transient but persist beyond the acute experience of breast

biopsy. This may be of particular relevance to women diagnosed with malignancy since they will be facing additional stressors related to cancer diagnosis and its associated treatment. Anderson *et al* (Andersen, B. L., Farrar, W. B., Golden-Kreutz, D., Kutz, L. A., MacCallum, R., Courtney, M. E., & Glaser, R., 1998) studied women with breast cancer who had recovered from the acute effects of their breast cancer surgery, but had not yet begun either chemo- or radiotherapy. They found that perceived stress predicted lower NKCA and responsiveness to IFN- γ in these women with breast cancer. The results reported herein provide evidence that stress-induced immune-dysregulation begins even earlier in the course of the breast cancer experience, at the time of impending breast biopsy.

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Table 1

Production of Th2 Cytokines.

Cytokine	Pre biopsy	N	Post biopsy	n	Normal Controls	n
IL-4	0.26±0.04 ^a	58	0.22±0.03	58	0.14±0.03	18
IL-10	4.75±0.74 ^b	61	4.78±1.01 ^a	56	1.32±0.18	28

Values represent mean ± S.E. in ng/ml of culture supernatant. Statistical comparison between experimental mean and control; ^a=p <0.05, ^b=p<0.01.

Table 2

Phenotypic analysis of PBMC.

Phenotype	Pre biopsy	N	Post biopsy	n	Normal Controls	n
CD56+	7.03±1.10	27	6.91±1.22	22	4.70±2.09	8
CD16+	6.18±0.46	27	6.85±0.65	22	5.79±1.05	8
CD56/16+	6.06±0.61	27	5.69±0.54	22	5.55±1.17	8
CD56/16+ & CD56+	13.09±1.56	27	12.60±1.60	22	10.25±2.97	8

Values represent mean ± S.E.

FIGURE LEGENDS

Figure 1. Psychological measures of perceived stress and of stress are depicted pre biopsy, post biopsy, and for non-biopsied, control women. Perceived stress was measured using Cohen's Perceived Stressor Scale (PSS). Stress pre and post breast biopsy was measured by use of 10 cm visual analogue scales that determined global stress (VAS). Bars represent the mean values \pm S.E. The numbers of subjects (N) in each group were: PSS pre=48, PSS post=45, VAS pre=48, VAS post=39, Control PSS= 45, Control VAS=25. Statistical comparisons are presented as follows for Figures 1 - 5. Statistical comparison between biopsied experimental mean values and normal control values; ^a=p <0.05, ^b=p<0.01, ^c=p <0.001. Statistical comparison pre to post for biopsied, non-malignant, women; ^d=p <0.05, ^e=p <0.01, ^f=p <0.001.

Figure 2. Psychological measures of mood state and anxiety are depicted pre biopsy, post biopsy, and for non-biopsied, control women. Mood state was measured using the Profile of Mood States (POMS) and the total mood disturbance (TMD) is depicted. Anxiety was measured using Spielberger's State Anxiety Inventory (STAI). Bars represent the mean values \pm S.E. N for; POMS-TMD pre=46, POMS-TMD post=46, Anxiety pre=48, Anxiety post=45, Control POMS-TMD=46, Control Anxiety=25. Statistical comparison between experimental mean and control; ^a=p <0.05, ^b=p<0.01, ^c=p <0.001. Statistical comparison pre to post; ^d=p <0.05, ^e=p <0.01, ^f=p <0.001.

Figure 3. The subscales of the POMS are depicted pre biopsy, post biopsy, and for non-biopsied, control women. N for; POMS-T pre=46, POMS-T post=45, POMS-D pre=46, POMS-D post=45, POMS-A pre=46, POMS-A post=45, POMS-V pre=46, POMS-V post=45, POMS-F pre=46, POMS-F post=45, POMS-C pre=46, POMS-C post=45, POMS-T Control=46, POMS-D Control=46, POMS-A Control=46, POMS-

V Control=46, POMS-F Control=46, POMS-F Control=46, POMS-C Control=46. Results for the vigor sub scale are presented as -(mean) for ease of presentation. Statistical comparison between experimental mean and control; ^a=p <0.05, ^b=p<0.01, ^c=p <0.001. Statistical comparison pre to post; ^d=p <0.05, ^e=p <0.01, ^f=p <0.001.

Figure 4. PBMC production of IL-2, IFN γ , and IL-6 are depicted for prebiopsy, post biopsy, and for non-biopsied control women. Peripheral blood was collected pre and post breast biopsy and from control women. PBMC were activated with PMA/PHA and culture supernatants were collected at 48 hr. Cytokine concentration was determined by ELISA. N for; IL-2 pre=36, IL-2 post=30, IL-6 pre=41, IL-6 post=38, INF γ pre=42, INF γ post=37, IL-2 Control=19, IL-6 Control=26, INF γ Control=21. Statistical comparison between experimental mean and control; ^a=p <0.05, ^b=p<0.01, ^c=p <0.001. Statistical comparison pre to post; ^d=p <0.05, ^e=p <0.01, ^f=p <0.001.

Figure 5. NKCA, expressed as lytic units at 20%, is illustrated for pre-biopsy, post biopsy, and non-biopsied control women. Peripheral blood was collected pre and post breast biopsy and from control women. NKCA was measured using K562 tumor cells as the target. N for; NKCA pre=47, NKCA post=48, NKCA Control=13. Statistical comparison between experimental mean and control; ^a=p <0.05, ^b=p<0.01, ^c=p <0.001. Statistical comparison pre to post; ^d=p <0.05, ^e=p <0.01, ^f=p <0.001.

Figure 1.

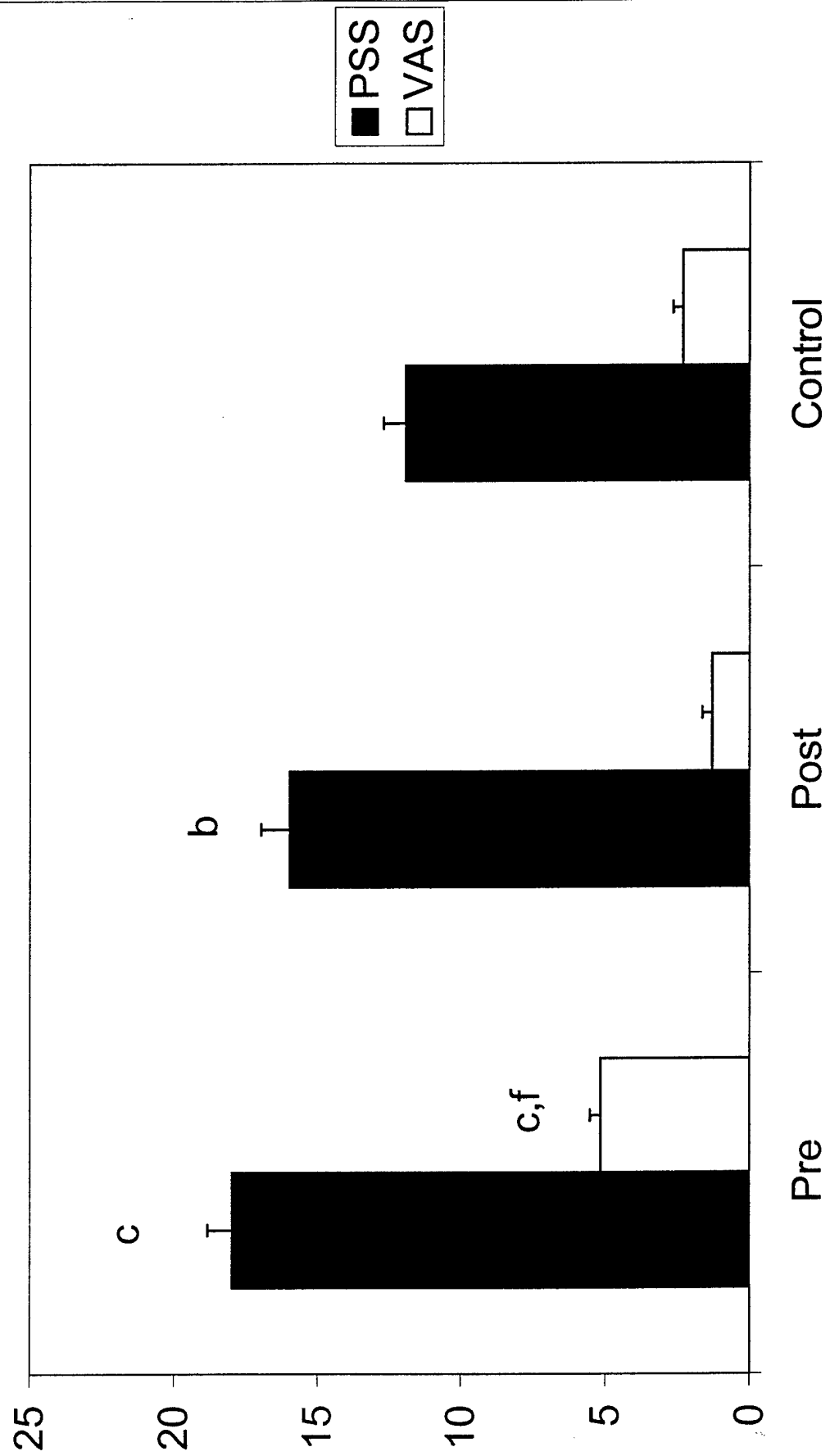


Figure 2.

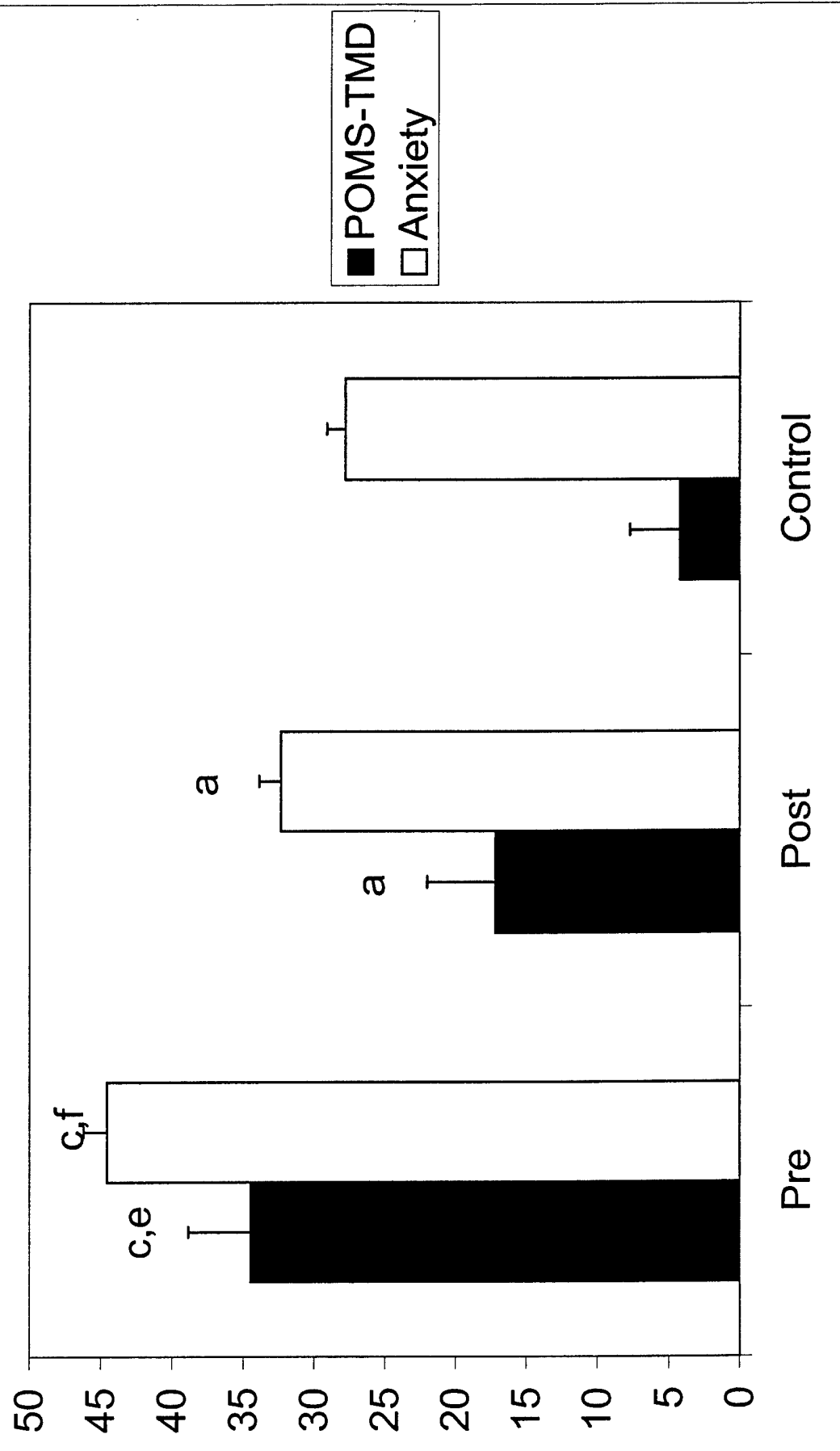


Figure 3.

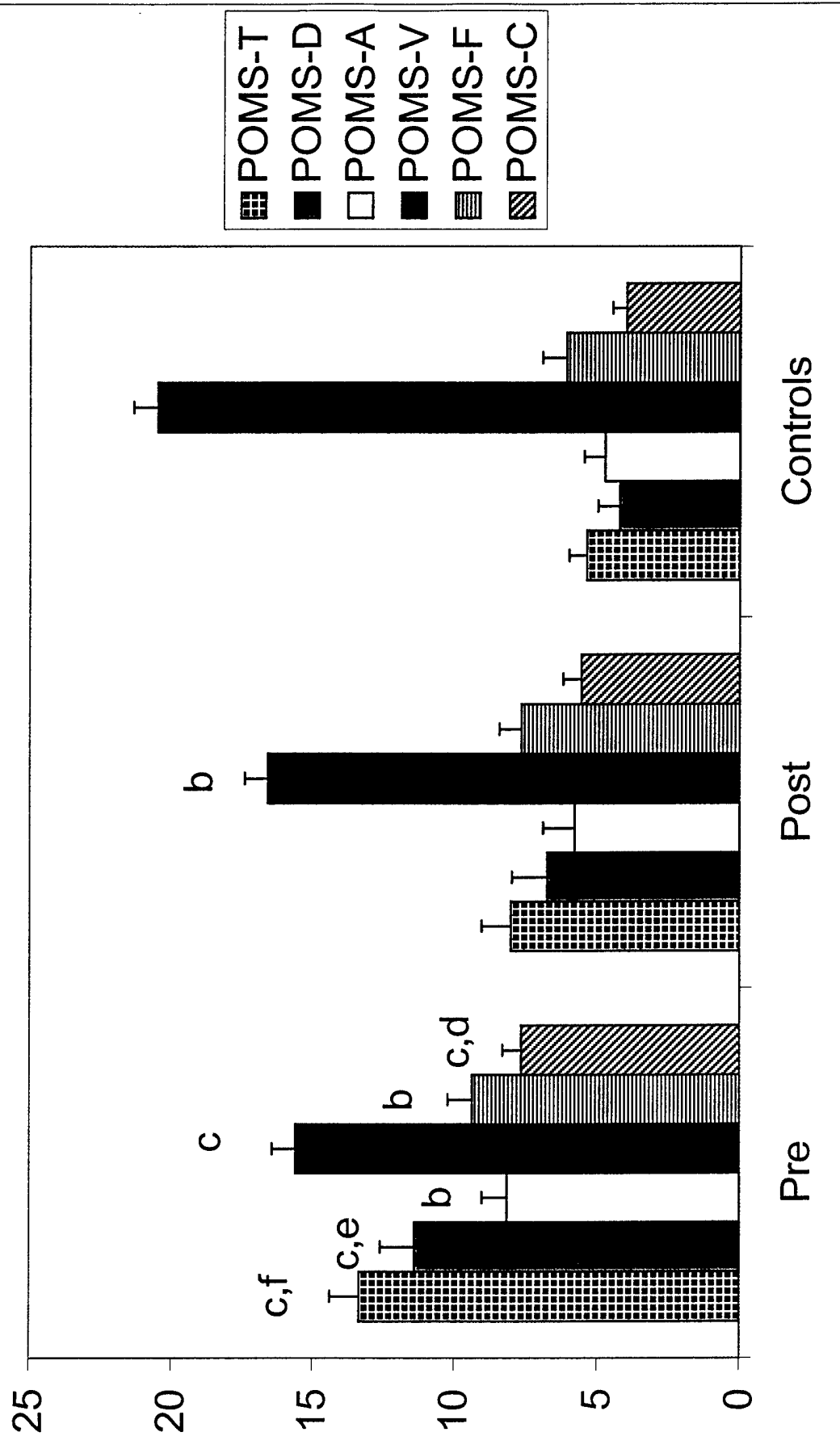


Figure 4.

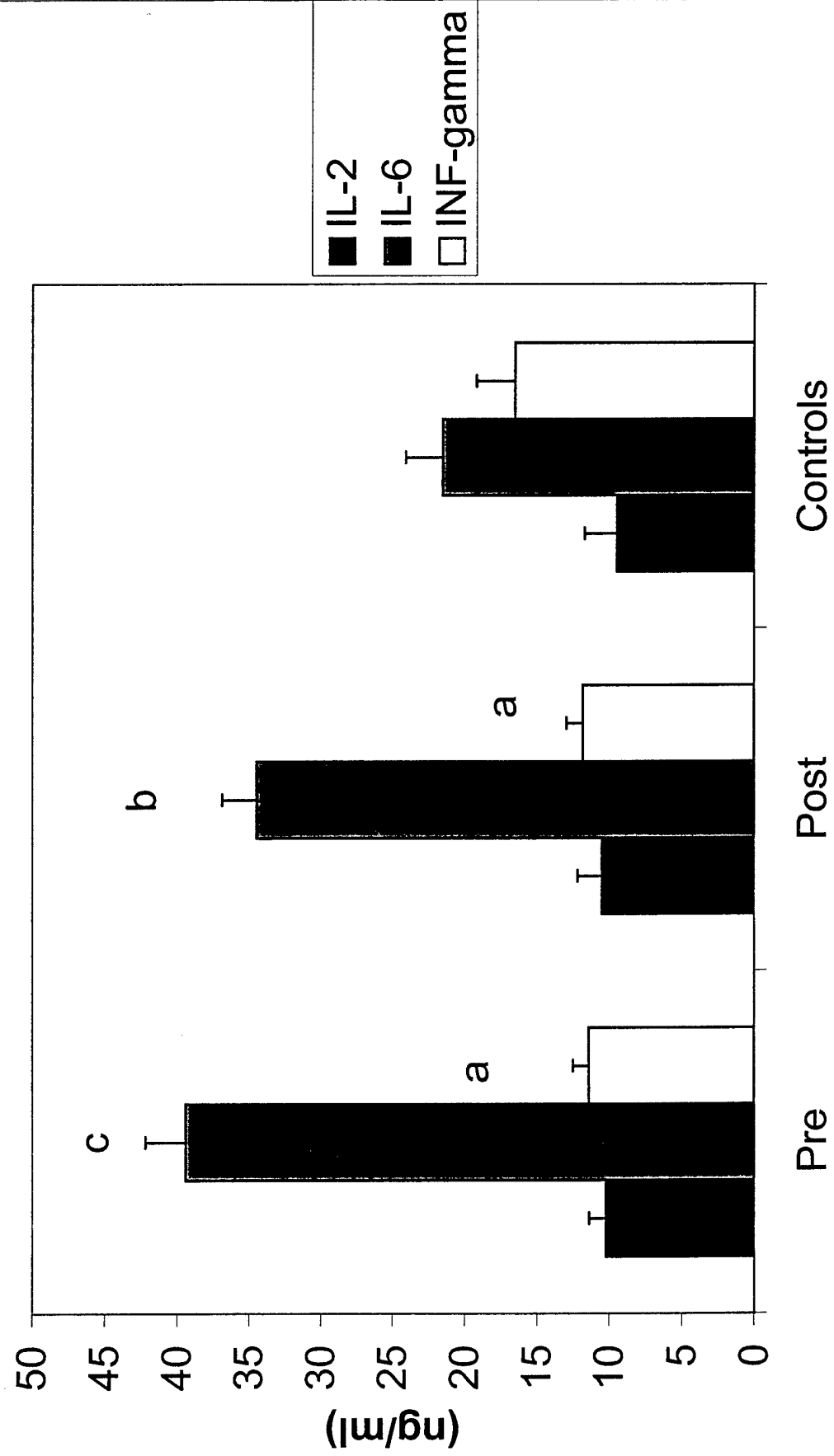


Figure 5.

